Poster session 2
Cell therapy / cellular therapy II

P381

Human cord blood endothelial progenitors directly and indirectly promote vascularization in immunocompetent hindlimb ischemia mouse model

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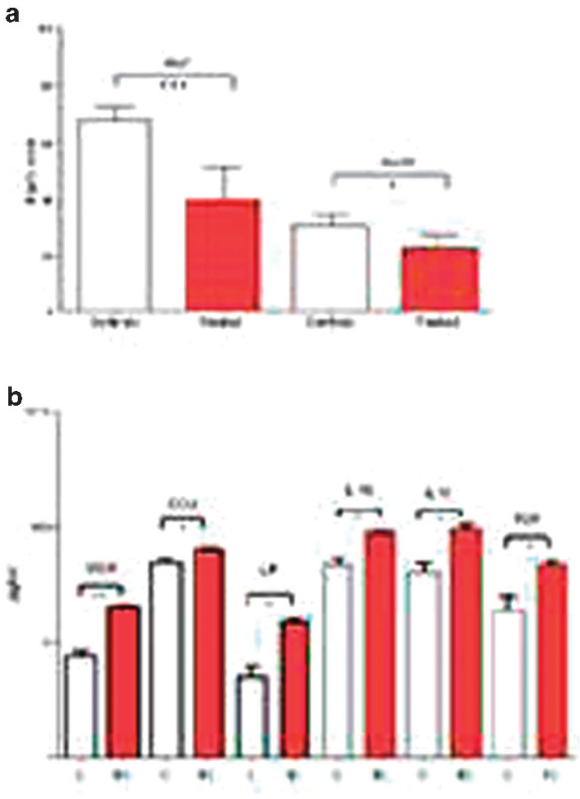
Introduction: Umbilical cord blood (CB) is particularly enriched with very immature endothelial progenitors (i.e. endothelial colony forming cells, ECFC) able to promote neo-vascularization in hind limb ischemia immunodeficient mice (Schwarz TM et al. Arterioscler Thromb Vasc Biol 2012;32:e136). We previously demonstrated that CB ECFCs show scarce HLA antigen expression (Nuzzolo et al. Blood Transfus. 2014, Suppl 1:s367-74) and do no express AB0 blood group antigens. Indeed, similarly to mesenchymal cells, also the utilization of CB ECFCs could be not HLA-restricted. Here, we evaluated the revascularization potential of CB ECFCs in hind limb ischemia mouse model utilizing immunocompetent recipients.

Materials (or patients) and methods: ECFCs were obtained according to Ingram et al. (Blood 2004;104:2752) and utilized after the third passage. Unilateral hind limb ischemia was induced in C57/BL6 mice as described (Biscetti F et al. Diabetes. 2010; 59:1496). Animals ($n = 20$) received intramuscular injection of CB ECFCs ($1x10^6$ cells in 50 mL of PBS) into the right thigh and calf. The control group ($n = 10$) received only PBS. The laser Doppler perfusion analysis was performed at weekly intervals for 28 days. At weeks1 and 4 after surgery, mice were sacrificed and tissues stained with hematoxylin-eosin to evaluate the muscle fiber integrity score (from 0, i.e. no injury, to 100, i.e. maximum injury) by counting 50 fiber/animal and scoring individual fibers as 0 (no injury), 1 (visible vacuolization), or 2 (disintegration) (Chan RK et al. Surgery 2006;139:236). The presence of CB ECFC injected cells was evaluated by immunohistochemistry using an anti-human CD31moAb (Dako). Plasma samples of sacrificed mice were analyzed for various angiogenic growth factors using the Bioplex pro-mouse cytokine panel at Bioplex cytomer (Bio-Rad).

Results: Overall, no limb loss was observed in treated and control animals. Nevertheless, at laser doppler analysis, the mean blood flow rate in ECFC treated mice was higher than in controls. The response was as early as day 7 and persisted until day 28 ($0.42 > 0.28$ at day 7, $0.63 > 0.42$ at day 14 and $1.10 > 0.82$ at day 28, respectively). Regarding inflammatory cell infiltration, no difference were found in samples obtained from treated and control mice. At days 7, and to lesser extent at day 28, treated mice showed significantly higher number of injured fibers than controls (Figure 1a). After 7 days from injection, several human CD31+ cells were detectable in treated animals, whereas lower amount of human CD31+ cells was detectable at day 28. Finally, we found that the ECFC injected mice exhibited a a higher secretion of VEGF, bFGF, IL18, CCL9 and LIF in response to ischemia (Figure 1b).



Conclusion: Overall, these data show that injected CB ECFCS were not rejected in C57/BL6 mice and contribute to revascularization and tissue repair both directly and indirectly, by stimulating the production of growth factors and chemokines in the recipients. Our findings support the utilization of CB ECFCs as allogeneic cell therapy product for untreatable critical limb ischemia. **Supported by Fondi di Ateneo UCSC 2014.**

Disclosure of Interest: None declared.

P382

A pilot study of post-transplant hypomethylating agents + Bortezomib + NK/gamma-delta T cell infusions in children with hematologic malignancies

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Introduction: Relapse of malignant disease remains the most important cause of failure after allogeneic transplantation for hematologic neoplasms in children. Toxicity is a major concern in preventive posttransplant therapy, particularly in the early months after AlloSCT. Last year we presented a pilot study of decitabine in children with hematologic malignancies after

allogeneic HSCT. In the current study we evaluated a combination of hypomethylating agents (decitabine or azacytidine) + bortezomib + Innate DLI- (PBSC depleted of of $\alpha/\beta(+)$T cells) in the same clinical setting.

Materials (or patients) and methods: Thirty one patients recieved 92 course between 27.02.2013 and 10.10.2014. Cohort included 24 boys and 7 girls, median age 7,6(0,76-21) years. Twenty patients had acute myeloid leukemia (CR1-12, CR2-1 CR > 2 -1, AD-6), 7 – acute lymphoblastic leukemia (CR2 - 6, AD-1), 2 – biphenotypic leukemia, 2 – juvenile myelomonocytic leukemia. Twenty three patients got uniform conditioning with total doses of treosulfan 42 g/m2, horse ATG (ATGAM) 50 mg/kg, melphalan 140 mg/m2, fludarabine 150 mg/m2. Eight patients, transplanted in 2014, received rabbit ATG (Thymoglobuline) 5 mg/kg, instead of horse ATG, rituximab 100 mg/m2, and bortezomib 5,2 mg/m2 (-5-2, + 2 + 5).

Nineteen patients were transplanted from matched unrelated donors, 12 – from haploidentical donors. All grafts were depleted of TCRalpha/beta T cells and CD19 B lymphocytes.

Hypomethylating agents + Bortezomib + DLI courses were planned to start at after day + 30 as soon as WBC reached > 1x103/l, ANC > 0,5x103/l, PLT > 30x103/l, Hb > 95 g/l. Courses included: Decitabine 10 mg/m2 or Azacytidine 35 mg/m2 (1-5days), Bortezomib 1,3 mg/m2 (2,5 days), DLI (product of negative depletion of $\alpha/\beta(+)$ T cells) (on day 6). Two-three courses were to be administered at 30-45 day intervals.

Results: Median time from HSCT to first course was 42 (31-163) days. Median number of courses was 2 (1-5). Hematologic toxicity included grade IV neutropenia in 4%, grade III in 21%, grade II in 21%; grade III thrombocytopenia in 3,3%, grade II in 17%. None of the patients recieved platelet or RBC transfusions. Hepatic toxicity included grade III ALT/AST elevation in 4,4%, grade II in 31%. Renal toxicity – transient grade I azotemia in 6,5%. Neuropathy I grade 28%. Neuropathy > grade II in 1 patient. aGVHD after DLI was observed in 6 pts (18,7%). Limited skin lesion was observed in 4 pts, visceral grade 2-3 in 2 pts. Infections complicated therapy in 18 patients. Overall grade I infections developed during 11% courses, grade II in 13%, grade III (requiring inpatient care) – in 4,4%.

Nine of the 31 patients experienced disease relapse, cumulative incidence of relapse being 36% (95%CI: 21-62). For those transplanted in AD relapse incidence was 46% (95%CI22-96) vs in CR relapse incidence was 24%(95%CI11-44). Relapse incidence in AML was 26% (95%CI: 10-66)

Conclusion: Combined therapy with hypomethylating agents, bortezomib and NK/gamma-delta T cell DLI can be safely administered post-transplant in the outpatient setting with minimal toxicity. The therapeutic value of this approach should be evaluated in prospective comparative study.

Disclosure of Interest: None declared.

P383

Cytomegalovirus specific T cells do not allo-react in the human-*in vitro* skin explant model

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Introduction: Adoptive T cell therapy represents an alternative approach to control virus reactivation in transplantation patients' refractory to anti-viral drugs. Despite improvement of antigen-specific T cell isolation techniques to date the safety of virus-specific T cell therapy has not yet been elucidated. The aim of this study was to investigate the allo-reactivity potential of Cytomegalovirus (CMV)-specific T cells via a human in vitro skin explant model (Skimune™).

Materials (or patients) and methods: CMV-specific T cells were isolated from 4-12x10^{7}periphereal blood mononuclear cells (PBMCs) from CMV seropositive healthy volunteers ($n = 10$) by in vitro stimulation with the specific CMV peptide pp65 and Large Scale Interferon (IFN)-ϒ Secretion Assay (Miltenyi). After in vitro expansion the purity of CMV-specific T cells was evaluated by short-term restimulation with pp65 followed by intracellular IFN-ϒ staining. The immunogenicity of the virus-specific T cells lines was analysed by Skimune™ in matched and mismatched virus-HLA-restricted individuals ($n = 4$) and these were compared to the alloreactivity potential of unselected PBMCs from the same CMV seropositive donor (positive control). Briefly, specific T cells were incubated with allogeneic PBMCs in a mixed lymphocyte reaction for 7 days followed by co-incubation with the skin tissue from the same donor. The skin was then assessed for histopathological damage and graded (I-IV) using graft-versus-host reactivity (GvHR) criteria. IFN-ϒ and Granzyme B levels were detected by the Cytometric Bead Array assay ($n = 4$).

Results: After stimulation of PBMCs with pp65 and immunomagnetic selection with the manual MACS separator a mean yield of 3.12 (± 0.98 SEM) x10^5 cells was obtained with a mean purity of 71.85% (± 5.4% SEM) for CD4 and 79.16% (± 6.27% SEM) for CD8 positive CMV-specific T cells. In terms of absolute number there was no difference between CD4 and CD8 positive isolated virus specific T cells. To further investigate the specificity and immunogenicity of CMV-specific T cells, isolated cells were expanded in culture between 10-28 days with a median of 142 fold increase in the cell number and a significant predominance of CD8 positive cells ($P = 0.017$). The specificity of the expanded CMV-specific T cells after-short term re-stimulation was 32.58% (± 5.77 SEM) and 74.68% (± 3.91 SEM) respectively for CD4 and CD8 positive T cells. The Skimune™ assay showed an absence of skin damage (Grade I: negative response) for all CMV-T cells highlighting their specificity and lack of cross-reactivity in a HLA mismatched system. A positive allo-reaction was observed in the corresponding positive controls where the skin showed a response between Grade II and III GvHR. The absence of an immune allo-response by virus-specific T cells was further confirmed by the low levels of IFN-ϒ (9.418 pg/ml ± 8.986 SEM) and Granzyme B (288.1 pg/ml ± 286.0 SEM) in the supernatants compared to the corresponding positive controls (respectively 709.4 pg/ml ± 393.5 SEM and (978.2 pg/ml ± 325.2 SEM).

Conclusion: Our work demonstrates that HLA-mismatched virus-specific T cells from a third-party donor can be safe and encourages the development of a virus specific T cell bio-bank for the rapid treatment of transplantation patients by adoptive anti-viral T cell immunotherapy.

Disclosure of Interest: None declared.

P384

A Multi-Antigen Specific T Cell Product for the Prevention of Viral Infections and Tumor Relapses Early after Allogeneic Stem Cell Transplantation

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Introduction: One of the major challenges in the field of allogeneic stem cell transplantation (alloSCT) is to induce graft versus leukemia (GVL) responses without coinciding graft versus host disease (GVHD). T cell depletion of the graft reduces the risk on acute GvHD, but renders patients vulnerable to viral infections and disease relapses. In this study (T-control, EU FP7), we aim to restore anti-viral immunity and support GVL responses with the prophylactic infusion of a multi-antigen specific T cell product, 6-8 weeks after T cell depleted HLA matched alloSCT. This product consists of donor T cells directed against HLA class I restricted peptides of CMV, EBV and adenovirus, the tumor associated antigens (TAA) WT1, RHAMM, NY-eso, PRAME and proteinase 3, and the minor histocompatibility antigen (MiHA) HA-1.

Materials (or patients) and methods: The reversible HLA/peptide streptamer technology and magnetic bead isolation allows selection of antigen specific T cells under Good Manufacturing Practice (GMP) conditions. High avidity memory virus specific T cells can be easily enriched from blood of seropositive donors. However, due to the extremely low precursor frequencies of TAA and MiHA specific donor T cells, and virus specific T cells from seronegative donors the isolation of these cells is more complicated. Our aim is to simultaneously isolate HLA-A2 restricted CMV, EBV, AdV, TAA and MiHA specific T cells from the memory as well as the naïve donor T cell repertoire. Additional virus specific streptamers for other HLA alleles will be added based on the donor's HLA type. The isolation is started with 2*10^9 donor PBMC and $\leq$16 different streptamers will be combined in one isolation.
Results: The GMP test runs ($n = 3$) resulted in a product consisting of 5-10*10^6 cells with a purity of 82-92% antigen specific T cells. As expected, virus specific memory T cells comprised the main portion of the product. To investigate whether the remaining part of the product contained TAA, MiHA and virus specific T cells from the naive compartment, we performed subsequent streptamer enrichments with these specific streptamers, followed by non-specific expansion. Using this strategy, all TAA, MiHA and virus specificities could be enriched to detectable frequencies after 2 or 3 isolations when starting with $\geq$500*10^6 donor PBMC, despite the initial invisible precursor frequencies. This demonstrates that these specific T cells are already an elementary component of the product after the first isolation procedure, indicating that additional culturing and expansion of the product before administration to the patient is not necessary. Therefore, the multi-antigen specific T cell product will be isolated and administered to the patient on the same day, making this therapeutic cell product a non-ATMP. Moreover, these data illustrate that combining multiple streptamers in one isolation procedure does not negatively influence the isolation of specificities present in undetectable frequencies in the starting material.
Conclusion: We are able to generate from donor PBMC a highly purified multi-antigen specific T cell product for direct clinical application. Besides the main component of virus specific CD8+ T cells from the memory compartment of the donor, we have demonstrated that this product also contains TAA, MiHA and virus specific T cells enriched from the naive compartment of the donor.
Disclosure of Interest: M. Roex: None declared, E. van Liempt: None declared, L. Hageman: None declared, L. Germeroth Conflict with: Lothar Germeroth is shareholder at Stage Cell Therapeutics which develops Cell therapeutics based on the Streptamer technology., C. Halkes: None declared, F. Falkenburg: None declared, I. Jedema: None declared.

P385
A pilot trial of low-dose infusions of CD45RA depleted donor lymphocytes to improve immune reconstitution after TCR alpha/beta depleted transplantation

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Introduction: Severe viral infections remain the most important cause of non-relapse mortality and morbidity in recipients of TCR-alpha/beta depleted grafts. Fully functional reconstitution of adaptive immunity is delayed until day $>+150$ in most patients. Although transfer of *ex-vivo* generated or CCS-selected virus-specific T-cells are established methods of therapy for severe viral infections, these procedures remain expensive and complicated. In this pilot trial we are testing the hypothesis that I infusions of memory T-cells are safe and can produce anti-viral responses.
Materials (or patients) and methods: Ninteen patients, median age 9y (2-18), m/f-11/8, with malignant (n- 14) and non-malignant (n-5) blood disorders recieved TCR-alpha/beta depleted transplants between 27.02.14 and 16.10.2014. Donors were haploidentical (n-12) and matched unrelated (n-7). Patients were eligible for participation if they had stable graft function, no signs of active GVHD or severe infection. All patients with malignant disease were in remission at time of transfusion. Only CMV seropositive donor/recipient pairs were eligible. Three infusions of memory T-cells were planned at escalating doses ($25x10^3$/kg, $50x10^3$/kg, $100x10^3$/kg in haploidentical, $100x10^3$/kg, $200x10^3$/kg, $300x10^3$/kg in MUD transplants), with monthly interval. Memory T-cells were derived from G-CSF stimulated (n - 14) or unstimulated (n - 5) apheresis of the original donors. Apheresis product was processed with single-step CD45RA depletion procedure on CliniMACS Plus (n - 19) instrument, according to the manufacturer's recommendations. Incubation volumes and reagent concentration were adjusted to the nucleated cell load. CD45RA-depleted fraction was aliquoted and cryopreserved for further use. Beyond routine monitoring of CMV, EBV and Adeno DNA load, lympocyte subset regeneration and hematopoietic chimerism, development of pathogen-specific (CMV, EBV, Adeno) immunity was monitored monthly by the ELISPOT assay for IFN-gamma production by PBMC in response to respective antigen stimulation.
Results: Between 15.04.14 and 28.11.2014 19 patients recieved 40 transfusions of memory T-cells. The procedure of CD45RA depletion was effective with $>$4,5 log depletion of CD45RA. Final product contained negligible numbers of CD45RA+ naive T-cells. Median day of the first infusion was +47 (12-77). At the moment of first "memory cell" infusion 15 (75%) patients had detectable CMV DNA in the blood. Median follow-up was 80 (21-204) days from first infusion. None of the recipients developed signs of GVHD $>$1 grade (grade 1 skin GVHD developed in 4 (21%) pt). Fifteen patients could be evaluated for generation of CMV-specific immune response. In these patients median number of CMV-reactive cells in the peripheral blood increased significantly from baseline: median 1,5 (0-650) to 171 (0-1143) cells per 3x105 MNC, Mann-Whitney p - 0,003. Of note, two patients were able to clear CMV without pharmacological intervention.
Conclusion: This preliminary analysis suggests that transfusions of low-dose memory T-cells after TCR-alpha/beta depleted transplants is a simple, safe and potentially effective method to improve post-transplant immune reconstitution in the T-depleted haploidentical and unrelated transplantation. This method should be tested prospectively as a prophylactic measure.
Disclosure of Interest: None declared.

P386
Human Platelet Lysate Promotes Proliferation and Maintains Immunomodulatory Capacity of Mesenchymal Stromal Cells

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Introduction: Due to the immunomodulatory properties of Mesenchymal Stromal Cells (MSCs), there has been increasing interest in the role of MSCs in allogeneic hematopoietic stem cell transplantation, especially in the prevention and treatment of graft-versus-host disease (GvHD). However, clinical applications for MSCs are hampered by the dependence on animal serum for MSC clinical expansion. In the past few years, many laboratories adapted their expansion protocols to human Platelet Lysate (hPL). Nevertheless, the effect of hPL on MSC biological functions remains to be elucidated.
Objectives: This study examines the feasibility of replacing FCS with human platelet lysate (hPL) for expansion of bone marrow (BM) derived MSCs by comparing growth/

morphology, phenotypic characteristics, tri-lineage differentiation and immunosuppressive properties.
Materials (or patients) and methods: BM derived MSCs were expanded in basal medium supplemented with 10% FCS (MSC-FCS) or 5% hPL (MSC-hPL). MSCs were characterised for immunophenotype, morphology/growth and tri-lineage differentiation capacity. The immunosuppressive capacity of MSCs was assessed by addition of TNFα and IFNγ primed or non-primed MSCs, at a ratio of 1:5, 1:10 or 1:20 MSC:TC, to mixed leucocyte reaction (MLR) consisting of CD3+ T cells and allogeneic monocyte derived Dendritic Cells. T cell proliferation was assessed by thymidine incorporation. The expression of CD69, CD25, Cutaneous Lymphocyte Antigen (CLA) and Programmed Death 1 (PD-1) on MLR primed T cells was assessed by flow cytometry. The ability of MSC-FCS and MSC-hPL to inhibit graft-versus-host reaction (GvHR) in the skin was assessed by the Skin explant assay, where MSCs with or without TNFα and IFNγ priming were added to MLR as stated above, followed by co-culture of the MLR cells and skin autologous to DCs. Tissue damage was evaluated according to Lerner criteria. Paired-T tests were used for statistical analysis with a *P*-value of ≤ 0.05 as significant.
Results: MSC-FCS and MSC-hPL exhibited similar characteristics in terms of phenotype, morphology, and tri-lineage differentiation potential. However, MSC-hPL showed higher proliferative capacity than MSC-FCS, exhibiting higher cumulative population doublings ($P<0.001$). MSCs efficiently suppressed T cell proliferation in a dose dependent manner, with an over 80% inhibition at highest MSC concentration. There was no significant difference in the potency of T cell proliferation suppression between MSC-hPL and MSC-FCS, regardless of IFNγ and TNFα priming. Compared to no MSC control, the presence of MSC in the MLR mediated a decreased expression of CD25, CLA & PD-1 and an increased expression of CD69 by alloreactive T cells ($P<0.05$ for all). This observation is consistent for both CD4 and CD8 T cells as well as for both MSC-FCS and MSC-hPL. Preliminary data from the skin explant assay did not show significant down regulation of cutaneous GvH tissue damage by either MSC-FCS or MSC-hPL at the current setting, but further work is ongoing to validate this finding.
Conclusion: The data suggest that hPL is a promising candidate to replace FCS for xeno-free MSC expansion. MSC-hPL retained classical MSC characteristics and immunomodulatory function with enhanced proliferation capacity.
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P387
Deciphering the Role of Mesenchymal Stem Cells Paracrine in Wound Closure Acceleration

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Introduction: Recently, mesenchymal stem cells (MSCs) gained attention as a therapeutic strategy for wound healing due to their paracrine secretions which may enhance keratinocyte migration and differentiation into the site of injury. Therefore, this research was conducted to investigate the role of MSC-conditioned medium (MSC-CM) in accelerating keratinocyte migration into the injury site.
Materials (or patients) and methods: MSCs were isolated from human bone marrow using standard protocols and characterised for morphologic, phenotypic characteristics and differentiation potential. When MSCs reached 80% confluence, their culture supernatants were collected in serum free medium (SFM) from different passages (1, 2 and 3) and after different time periods (24 hr, 48 hr and 72 hr). For example, MSC supernatants collected from passage 1 after 72 hr are referred to as CM-P1-72, MSC supernatants collected from passage 2 after 48 hr are referred to as CM-P2-48 and MSC supernatants collected from passage 3 after 24 hr are referred to as CM-P3-24. The effect of these MSC-CM on migration of a human keratinocyte cell line (HaCat) was evaluated using a 2D scratch assay.
Results: Isolated MSCs met the minimal criteria as stipulated by the International Society for Cell Therapy (ISCT) by being fibroblast-like cells with plastic adherence and capable of proliferating under *in vitro* conditions. Phenotypically, over 95% of the cells expressed CD73, CD90 and CD105 ($P=0.0001$), while being negative for the expression of CD14, CD19, CD34, CD45 and HLA-DR ($P=0.025$). Additionally, all cells demonstrated the ability to differentiate into three lineages; osteoblast, adipocyte and chondrocyte.
In 2D scratch assays, MSC-CM from different passages significantly enhanced void closure of HaCat keratinocytes. For example, MSC-CM-P1-72 was the most effective at promoting wound closure after 17 hr of treatment ($P=0.0009$). In addition, both MSC-CM-P2-48 and MSC-CM-P3-24 significantly reduced scratch areas after 17 hr of treatment ($P=0.0011$).
Conclusion: Keratinocyte mobility is a main mechanism which could be utilised to promote chronic wound healing. The scratch assay is a simple inexpensive technique which could be applied *in vitro* to monitor keratinocyte migration after treatment with pharmacological agents or test substances such as growth factors. Collectively, these results suggest that MSC-CM contain effector molecules required for cell migration in the wounded region i.e. it is reported that there are 36 cytokines released by human MSCs which act as key factors in wound healing. A possible explanation for promoting keratinocyte movement into the scratched area is that MSC-CM contain cytokines which cause accelerated cell migration such as nerve growth factor (NGF), IL-6, IL-8 and stromal derived factor-1 (SDF-1). Additionally, MSC-CM might contain other cytokines which can promote both keratinocyte migration and proliferation such as hepatocyte growth factor (HGF), platelet derived growth factor (PDGF) and granulocyte-macrophage colony stimulating factor (GM-CSF).
Disclosure of Interest: None declared.

P388
Placenta-Derived Decidual Stromal Cells for Graft-Versus-Host Disease, Hemorrhaging, and Toxicity after Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Mesenchymal stem cells (MSCs) may cure life-threatening acute GVHD, but long-term survival has been unsatisfactory. The placenta protects the fetus from the mother's immune system, and placental tissues have been used for over 100 years in Africa to successfully treat burn injuries. Decidual stromal cells (DSCs) are accessable without any invasive procedure, with little or no need for ethical considerations, as the placenta is normally discarded after delivery.
Materials (or patients) and methods: We isolate, culture, expand, and store DSCs from term placentas. They have fundamental differences to MSCs. DSCs do not differentiate as well to bone or cartilage. DSCs need cell-to-cell contact to be immunosuppressive and to induce FoxP3 regulatory T-cells. Blocking of the activity of IDO, prostaglandin E2, PD-L1, and interferon impairs the immunosuppressive capacity of DSCs in MLC. Human DSCs inhibit MLC in mice. MCSs and DSCs induce xenoreactivity. We have treated 43 patients with 136 doses of

DSCs following allogeneic hematopoietic stem cell transplantation (ASCT).
Results: In culture, DSCs can be expanded appreciably, and from one placenta we can obtain enough cells to treat more than 20 patients. With early treatment (within median seven days) of steroid refractory acute GVHD, response rate was 100% and one-year survival was 76%—as opposed to 6% in retrospective or time overlapping controls not treated with stromal cells ($P<0.001$). A partial response was seen in two of three patients with severe chronic GVHD. Four patients have been treated for hemorrhagic cystitis, and now we have proceeded with a prospective double-blind randomized study. A 33-year-old man developed acute respiratory distress syndrome (ARDS) after septicemia and ASCT. He required 15 L/min oxygen by mask. After infusion of 1×10^6 DSCs, oxygen saturation increased instantly from 92% to 98%; requirement for oxygen decreased, and it was discontinued after five days. Chest radiography improved and elevations in cytokines/chemokines, G-CSF, IL-6, IL-8, MCP-1, and TNF-alfa decreased. The patient is now alive and well ten months after ASCT. We reversed paresis in the arms and legs (respectively) of two patients with polyneuropathies. The first patient had recurrence of paresis and needed additional infusions of DSCs. The second patient has no symptoms four months after infusion. In a toxicity study survey of hospital charts, laboratory values, and autopsies, we found no major side-effects. Mild, spontaneously reversible acute side-effects were seen during three DSC infusions.
Conclusion: DSCs is an effective therapy for acute and chronic GVHD, hemorrhagic cystitis, ARDS, and neuropathy. So far, we have not seen any major side-effects.
Disclosure of Interest: None declared.

P389
Complemetary activity of NK and γδ T cells post TcRab/CD19 depleted haploidentical hematopoietic stem cell transplantation

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Introduction: TcRab/CD19 depletion for haploidentical hematopoietic stem cell transplantation has extended the number and type of effector cells post haploidentical stem cell transplantation. In the early posttransplant phase a γδ T cell wave comparable to the NK cell wave facilitates antitumor activity of γδ T cells aside with NK cells [1].
Materials (or patients) and methods: PBMCs of 2 healthy volunteer donors and 5 patients who underwent haploidentical HSCT (TcRab/CD19 depleted grafts) were challenged in a flow cytometric CD107a assay combined with intracellular staining for IFNγ. Further a flow cytometric based kill assay with CD69 assessment was performed. NALM-16 was used as target cell line with and without the Fc-optimized CD19 antibody 4G7SDIE (kindly provided by Gundram Jung). In all experiments effector to target ratio was, PBMCs to NALM-16, 1:1. IL2 and zoledronic acid was used to stimulate effector cells.
Results: PBMCs of healthy donors and patients showed a moderate cytolysis ($n_d=3$, $mean_d=26.9\%$; $n_p=5$, $mean_p=21.6\%$) and excellent antibody-dependent cellular cytotoxicity ($mean_d=47.8\%$, $mean_p=43.5\%$). CD107a, CD69 and IFNγ were significantly increased by NALM-16 coincubation. Besides on NK cells, CD19 antibody significantly increased CD107a expression in healthy donors and patients ($n_d=3$, $p_d=0.0014$; $n_p=7$, $p_p=0.0017$) as well as CD69 positivity ($n_d=3$, $p_d=0.15$; $n_p=7$, $P=0.0008$). The same was found in the γδ T cell compartment without antibody ($n_d=3$, $p_d=0.09$; $n_p=9$, $P=0.01$) and with CD19 antibody $p_d=0.09$, $n_p=0.002$). No difference between healthy donors and patients with regard to CD107a and IFNγ positivity with and without CD19 antibody as well as NK cell mediated cytotoxicity with and without CD19 antibody. Yet, there was a significant difference for CD69 positivity between healthy donor and patients with and without CD19 antibody. IL2 increased CD69 in NK and γδ T cells, whereas zoledronic acid led to increased CD69 positivity in γδ T cells only.
Conclusion: After TcRab/CD19 depleted haplo HSCT NK cells and γδ T cells exert complementary antitumor activity that can be significantly increased by FcgIIIa recruiting therapeutic antibody 4G7SDIE. No functional impairment of posttransplant NK and γδ T cells in comparison to healthy donor cells were revealed in the context of target recognition measured by cytokine production and CD107a as well as CD69 positivity, direct cytolysis and antibody-dependent cytolysis. Clinical application of IL2 and zoledronic acid might enhance NK and γδ T cell function in TcRab/CD19 depleted haploidentical stem cell transplantation.
References: 1. Oevermann, L., et al., *Immune reconstitution and strategies for rebuilding the immune system after haploidentical stem cell transplantation*. Ann N Y Acad Sci, 2012. **1266**: p. 161-70.
Disclosure of Interest: None declared.

P390
JCV-specific T-cell therapy can successfully treat progressive multifocal leukoencephalopathy

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Introduction: Progressive multifocal leukoencephalopathy (PML) is a rapidly progressive, often fatal, demyelinating disease of the central nervous system, developing in immunocompromised hosts as the result of JC polyomavirus (JCV) reactivation. Although rare, PML is increasingly being observed in transplant recipients, in patients treated with targeted therapies for cancer or autoimmune disorders, and even in apparently healthy individuals. Therapeutic options in PML have very limited efficacy. However, immune reconstitution has been claimed to lead to resolution of PML. Transfer of autologous or donor-derived JCV-specific T cells could restore virus-specific cellular immunity and control PML.
Materials (or patients) and methods: We conducted scale-up experiments to validate a GMP method for expansion of JCV-specific T cells from 6 healthy HSCT donors and 4 patients with PML, by peripheral blood mononuclear cell (PBMC) stimulation with 15-mer peptide pools derived from the JCV Viral Capsid (VP) 1 and Large T (LT) proteins. The lines were tested by ELISPOT assay and cytotoxicity analysis.
Results: T-cell lines (TCL) were successfully generated from 3 of 4 patients, and from 7 of the 8 HSCT donors. The TCL obtained from the patients included a majority of CD8+ T cells (median and range: 57%, 18-80) with 25% CD4+ T cells and 7% CD3-CD56+ NK cells (range: 1-80; 2-14, respectively), while those expanded from HSCT donors were mostly CD4+ T cells (median and range: 76%, 29-89), with 13% and 4% CD8+ T cells and CD3-CD56+ NK cells. The lines from both patients and donors included VP1- and LT-specific IFNg-producing cells (median frequency patients: VP1, 1565 SFU/10^6 cells; LT, 1400 SFU/10^6 cells. donors: VP1, 827 SFU/10^6 cells; LT, 718 SFU/10^6 cells). Five of the 8 TCL obtained from the donors exerted specific cytotoxicity above 10% (median lysis 21% at an effector to target ratio of 10:1), while only 1 of 4 TCL expanded from patients yielded a JCV-specific lysis >10% (25% at an effector to target ratio of 10:1). Two of the TCL (1 haploidentical and 1 autologous) were employed as therapeutic agent for PML, developed in 1 case after rituximab treatment for NHL and in the other case in an apparently healthy subject who revealed a idiopathic CD4+ T cell deficiency. The patients, who had progressive disease at the time of T cell therapy, received 4 infusions of small numbers of TCL (0.1 x 10^6 cells/kg bw per dose), in order to avoid further neurologic impairment as a consequence of IRIS. No adverse

event attributable to TCL infusion was recorded, and both patients showed a prompt clinical response, with improvement at mini-mental-state evaluation, and reduction of PML lesions on MRI imaging at a median follow-up of 5 months.
Conclusion: T cell therapy with JCV-specific TCL is an attractive and promising option that may restore virus-specific cellular immunity and cure immune deficient patients with PML.
Disclosure of Interest: None declared.

P391
Cell therapy with CD34/CD133 enriched in avascular necrosis of femoral head

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Introduction: Endothelial progenitor cells (CD 133 +), originated in the haemopoyetic lineage, are extensively researched for their high proliferative potential, and may have a role in the development of neoangiogenesis, although this function remains still largely unknown. Their selection from mobilized peripheal blood contains mesenchymal stem cells, also with proliferative competency, being able to differenciate into osteocytes and chondrocytes in appropiate conditions. In the present study we introduce CD 133 positive cells as a celular theraphy for avascular necrosis of femoral head (ANFH).
Materials (or patients) and methods: Six patients (aged between 20–54) with ANFH (Ficat stages 1-3) were treated with CD133 + cells (Table 1). Granulocyte colony stimulating factor was used for mobilisation to peripheal blood, dosed at 5 μg/kg for 5 days. The day before the surgery a leukapheresis procedure followed by a positive selection isolates CD133 + cells. CD133 positive and negative samples were analysed by flow cytometry. Cells were preserved 24 hours with autologous plasma and continuous agitation at 22°C. CD133 + were injected by percutaneous perforation in the necrosis área during the surgery. Patients 3 and 4 received two injections separated 18 and 10 months respectively. Osteonecrosis evolution was measured by magnetic resonance imaging (MRI), before the procedure, at 6 and 12 months.
Results: Following CD 133 treatment patients 1 and 6 showed radiological improvement with dissapearance of the necrotic areas described in the baseline MRI. They had stage 1 and 2 measured by Ficat classification before procedure and surgical approach was unilateral. Otherwise patient 3 and 4 showed no effects in the radiological images. A second perforation was performed in both cases and neither showed improvement. Their Ficat stage was 3 for both cases, and patient 4 had bilateral involvement, so the procedure was repeated 10 months after in the left femoral head. Patients 2 and 3 also showed no effect on MRI, Ficat stage was 3, and involvement was bilateral in patient 2, receiving treatment simultaneously in both femoral heads, and unilateral in patient 3.
Conclusion: CD133 + cells induced osteonecrosis recovery in patients with mild stages (Ficat 1-2), however there wasn't improvement in advanced stages. The data suggests that this therapy could be effective in early stage ANFH, but is difficult to determine in such a small study, so it must be performed on an increased number of patients. The role of bone marrow as a better source for this therapy, the optimal cell dose and additional research to produce a lasting clinical advantage has to be further investigated.
Disclosure of Interest: None declared.

P392
Combinational cell therapy for stroke: Bone Marrow Mono nuclear Cells plus Adipose tissue derived Stem cells improve muscles power after stroke

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Introduction: Stroke remains the main cause that transforms patients permanently disabled. At the time there are no known treatments for reversing this disorder. The use of autologous bone marrow derived mononuclear cells (MNCs) has been documented as treatment in such disorder; however, this treatment has limited results. Transplanted cells may encourage healing due to their effect on neighboring cells. To overcome this limitation, we have used a combination of adipose derived stem cell (ADSC) and MNCs in 4 doses, intrathecally, into adult rats.
Materials (or patients) and methods: Experimental model of cerebral ischemia have been developed in 12 adult rats (200 ± 20 g) to mimic human stroke. Group 1 (3 animals) was treated with 4 doses of MNCs (10^8 cells / dose), Group 2 (3 animals) was treated with 4 doses of ADSC (10^8 cells/ dose). Group 3 (9 animals) was administered a combination of both cell types at each dose. All doses were administered intrathecally at a time interval of four weeks.
Results: Subjects were assessed for improvement in the muscle power, movement behavior and overall functional ability. We have shown measurable reduction in movement disability. It was observed that treatment with a combination

[P391]

# PATIENT	AGE (years)	GENDER	INFUSION	FRACTION	VOLUME (ml)	NC x10⁶	CD133+ %	CD133+ x10⁶	CD34+ %	CD34+ x10⁶
1	54	Male	1	Apheresis	118	295	0.36	125	0.49	171
				CD133+ enriched	86	1.2	89	70.3	90	71
2	19	Male	1	Apheresis	136	290	0.35	138	0.43	170
				CD133+ enriched	69	2.2	86	131	97	149
3	61	Male	1	Apheresis	100	319	0.23	74	0.3	86
				CD133+ enriched	68	1.7	34	40	36.2	42
			2	Apheresis	140	311	-	-	-	-
				CD133+ enriched	150	0.8	*	*	*	*
4	34	Female	1	Apheresis	110	447	0.32	157	-	-
				CD133+ enriched	70	30	90.54	190	90.56	1874
			2	Apheresis	108	737	0.32	255	0.39	309
				CD133+ enriched	65	1.9	90.94	112	91.05	112.44
5	23	Male	1	Apheresis	100	19.7	-	-	-	24
				CD133+ enriched		*	*	*	*	*
6	37	Male	2	Apheresis	132	532	70.22	-	-	-
				CD133+ enriched	65	24	0.156	94.2	84.64	117.7

Table 1. Characteristics of patients and infused CD133+ fractions. NC : nucleated cells, *Due to technical problems Flow Cytometry analysis couldn't be performed.

Behaviors	Decrease in aggression	Anxiety	Sleeping pattern	Irritability
MNC	-	-	-	-
ADSC	-	-	-	-
Combination	++	++	++	++

cell therapy caused increase in muscle power and improvement in behavior at a faster rate than that achieved by MNCs alone.

Conclusion: Our result is a preclinical evidence to prove that combination cell therapy with ADSCs & MNCs is safe and efficient in enhancing treatment rate. Of course extensive research will have to be carried out before this claim can be made as a technique for cell therapy.

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P393

Adenosine Deaminase (ADA) deficiency: children's hospital of Brescia experience in bone marrow transplantation

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Introduction: Adenosine Deaminase (ADA) deficiency is an autosomal recessive immunodeficiency syndrome. ADA is an enzyme of purine salvage pathway that catalyzes the conversion of adenosine (Ado) and 2'-deoxyadenosine (dAdo) to inosine and 2'-deoxyadenosine, respectively. The lack of ADA cause the accumulation of Ado and dAdo, that can't be degraded, collected in plasma and transported into cells where they reach high levels.

These are toxic for immature lymphocytes, which thus fail to mature. As a result, the immune system is severely compromised or completely lacking. Patients with this defect typically present in the first months of life with recurrent infections, lymphopenia, failure to thrive and pneumonia. Candidiasis and persistent diarrhea are common. Various neurological disorders have been reported. These patients required decisive therapeutic protocols to reconstitute the immune function. There are 3 type of treatment: bone marrow transplantation (BMT), enzyme replace therapy with Peg-ADA and gene therapy.

Materials (or patients) and methods: After screening enzymatic test and confirmation of high levels of toxic metabolites, molecular diagnosis are performed on DNA samples. In our center 27 ADA patients and 6 new mutations were found, 10 undergoing BMT, 6 in treatment with Peg-ADA and 11 are in treatment in another center. 6 of BMT patients receiving marrow from HLA-identical siblings; the other 4 undergone transplantation with marrow from HLA-matched unrelated donor. All patients are constantly monitored for quantify toxic metabolities (Adenosine and dAdenosine) in red blood cells, evaluated for engraftment on peripheral lymphocyte subsets (assessed by STR based kit), lymphocytes counts and response to PHA.

Results: All BMT patients except one are alive and healthy. Pt 8 in fact, arrived at our center in serious conditions. He was moved to intensive care where he received a transplant to try to improve the clinical situation. Unfortunately the precarious conditions led to death only one week after transplantation. For the other patients engraftment is almost full donor on T cells and partial on B cells. 4 of our patients show complete engraftment on all subsets. Follow up range between 2 to 18 years. Lymphocyte's counts improve in 2-3 months after bone marrow transplantation and response to PHA was normalized between 5 and 17 months. These patients show normal level of Ado (av. 1.409 micromol/ml RBC) and lightly raise levels of dAdo (av. 0.033 micromol/ml RBC).

Conclusion: Recovery of immune competence has shown increased lymphocyte count and improvement of T lymphocyte function, few weeks after beginning treatment and it's maintained in the time. For our transplanted patients the time required for complete detoxification varies from 4 months to one year. In some cases the detoxification is not complete ma it's sufficient to have an increase in lymphocyte count and function. The response to mitogens in fact improves and normalize in few months. The bone marrow transplantation with HLA-identical sibling or HLA-matched unrelated donor could have risks related to the procedure but remained the treatment of choice where donor is available.

Disclosure of Interest: None declared.

P394

Purine Nucleoside Phosphorylase (PNP) deficiency: diagnosis and treatment

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Introduction: Purine Nucleoside Phosphorylase (PNP) deficiency is a rare autosomal recessive metabolic disorder which represents about 4% of all SCID. PNP catalyzes the reversible phosphorolysis of inosine, guanosine, deoxyinosine and deoxyguanosine. Physical finding are compatible with a history of recurrent infections and may include hepatomegaly, splenomegaly and developmental retardation; autoimmune disorders are also frequent and include autoimmune hemolytic anemia, idiopathic thrombocytopenia purpura, autoimmune neutropenia, systemic lupus and central nervous system vasculitis.

Variation in *clinical* presentation may cause difficulties in initial classification.

[P393]

Pt	BMT Type		CD34+/Kg x10^6	CD3+/Kg x10^6	Conditioning regimen	Follow up (years)	% of donor at last Engraftment			
							PBL	CD3+	CD15+	PMN
1	HLA-ID sister	BM	8.36	42.8	NO	10.4		100	94.8	0
2	HLA-ID brother	BM	7.6	52	NO	17.9		100	100	27.3
3	MUD	BM	12.6	23	BUS+EDX+ATG	11.5		100	100	19.3
4	MUD	BM	9	50	BUS+CICLOF+TIMOGL	8.2	100			100
5	HLA-ID sister	BM	3.8	16.93	NO	12	100			100
6	HLA-ID brother	BM	8	75	NO	12		96.1	81.7	63.2
7	MUD	BM	17.65	64	NO	5.3	100			100
8	HLA-ID sister	BM	14.73	104.35	NO	0.3	died			
9	HLA-ID brother	BM	18.36	47.5	NO	3		86.5		
10	MUD	BM	10.5	75.5	BUS+FLUDARA+ATG	2	100			100
			Average: 11.08	Average: 55.11						

Materials (or patients) and methods: 8 patients arrived at our center with suspected symptoms of PNP deficiency. Enzymatic and molecular diagnosis were performed in our lab. Spectrophotometric assay monitor the conversion of inosine to uric acid in presence of xanthine oxidase in RBC lysates. For 2 of these, enzymatic diagnosis wasn't possible because they were transfused.
5 patients showed borderlines values that suggested a possible transfusion not reported, only 1 patient (M.L.) showed zero enzymatic activity.
To confirm or exclude PNP deficiency, molecular diagnosis has been performed for all patients.
Results: M.L. was confirmed as PNP deficit, with a homozygous mutation called E89K due to a single base exchange, 3 patients showed polymorphism but no disease causing mutation and 4 not showed mutation.
4 patients continued to be followed at our center.
Three were diagnosed after: B.G. has been identified as SCID T-B + NK- gamma chain, M.E. bone marrow aplasia and T.H. primary immunodeficiency. All three have undergone bone marrow transplantation.
PNP deficit (M.L.) underwent allogeneic bone marrow transplantation from unrelated donor 4 months after diagnosis (Table).
Before transplant M.L. presented delay in psychomotor and language development and a progressive clinical deterioration with the appearance of a medium degree hemiplegia on the left side due to a venous thrombosis probably infectious based.
He received 15x10^6 CD34 + /Kg and 25x10^6 CD3 + /Kg. The conditioning regimen was Busulfan, ATG and Cyclophosphamide.
He presented hepatic and cutaneous GVHD II grade after transplant. Hemiplegia was stable.
110 months after BMT the patient remained free of infectious, complications and there was no signs of GVHD. Engraftment is complete for all subsets and the enzymatic values of PNP is in normal range.
Actually there's no neurological deteriorations and the patient has a partial recovery of the left side functionality.
Conclusion: Based on our experience in a single patient, allogenic BMT in PNP deficiency not correct neurogical abnormalities but may offer the possibility of correcting the immunodeficiency and preventing further neurological deterioration.
PNP-deficiency is extremely rare disease, less than 100 patients have been diagnosed and the symptoms are similar to other immunodeficiencies.
Disclosure of Interest: None declared.

[P394]

Patient	Enzymatic Values (healthy >300U/gHb)	Mutation	Age at BMT (months)	Type of BMT	Follow up (months)	Last Engratment
B.G.	213.5	NO	8	HLA-identical siblings	49	CD3+: 100% CD15+: 0%
C.G.	Not performed (transfused)	NO	patient returned to his center after exclusion of PNP deficiency			
F.A.	284	Polymorphism c.60C>T	patient returned to his center after exclusion of PNP deficiency			
M.E.	Not performed (transfused)	Polymorphism c.151C>A	103	Matched unrelated donor	79	PBL: 100% PMN: 100%
S.D.	255	NO				
T.H.	269	NO	32	Haploidentical	Deceased after 1 month	CD3+: 0% CD15+: 0%
T.G.	310.5	Polymorphism c.60C>T	patient returned to his center after exclusion of PNP deficiency			
M.L.	0	Polymorphisms c.151C>A c.171C>T Mutation: c.265G>A p.E89K	26	Matched unrelated donor	110	PBL: 100% PMN: 100%

P395

Isolation of clinical grade cord blood regulatory T cells using reversible streptamer technology

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Introduction: Haematopoietic stem cell transplantation (HSCT) is currently used to treat haematological malignancies and disorders, with delayed immune reconstitution, infection and graft versus host disease (GvHD) being the main complications. A number of studies have investigated the possibility of using regulatory T cells (Tregs) as a cellular therapy for GvHD, and of particular interest is third party cryopreserved umbilical cord blood (CB) as a possible "off-the-shelf" cell source, as CB is better tolerated in HSCT. However, previous clinical studies have focused on expanded CB Tregs; to date, selection from cryopreserved whole CB units, with sufficient numbers, purity and viability to allow for a minimally manipulated (non-ATMP) clinical product has been practically difficult. Here we follow development of a research grade Treg isolation protocol through to scale-up and pre-GMP (good manufacturing practice) compliance.
Materials (or patients) and methods: A streptamer (STAGE) based Treg isolation protocol was developed to isolate first from small scale $<400x10^6$ total nucleated cells (TNC), then from large-scale ($>500x10^6$ cells) and then using GMP compatible CB processing sets and DynaMag CTS magnet (life sciences) from cryopreserved units. For a comparison, adult Tregs were isolated, using a similar method, from freshly isolated CD4 + cells.
Results: As the protocol was developed from small-scale to pre-GMP, the purity of CD4 + CD25 + CD127lowFOXP3 + Tregs of the CD4 + (and CD3 +) population was maintained with fresh small-scale being median 89% of CD4 + cells ($n = 9$), small-scale cryopreserved ($n = 8$), 92%, large-scale cryopreserved ($n = 2$), 84% and pre-GMP large-scale ($n = 3$) 90%. However, it was also clear that it was difficult to maintain the levels of CD3- contaminants; median CD3- cell contamination of 19% for small-scale (combined fresh and cryopreserved), and increasing to 31% and 35% for large-scale and large-scale pre-GMP, respectively. The final pre-GMP yield indicated that from an average cryopreserved CB unit of

1.5-2.0x10^9 TNC one might expect 1-2x10^6 Tregs. Whilst the isolated cells showed good viability and were able to expand, current data suggests that streptamer isolated CB Tregs show weaker suppression compared with adult Tregs.

Conclusion: The loss of CD3- purity emphasises the need to have a clearly defined, but realistic, clinical product based on safety criteria during the development process. These results, however, demonstrate that a reasonable number of viable and functional Tregs, with a high purity of CD4+, can be isolated using a minimal manipulation method from cryopreserved CB units, which could potentially be used for cellular therapy in HSCT.

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P396

Survival after Mesenchymal Stromal Cell therapy in Steroid Resistant Acute Graft-Versus-Host-Disease. A systematic review and meta-analysis

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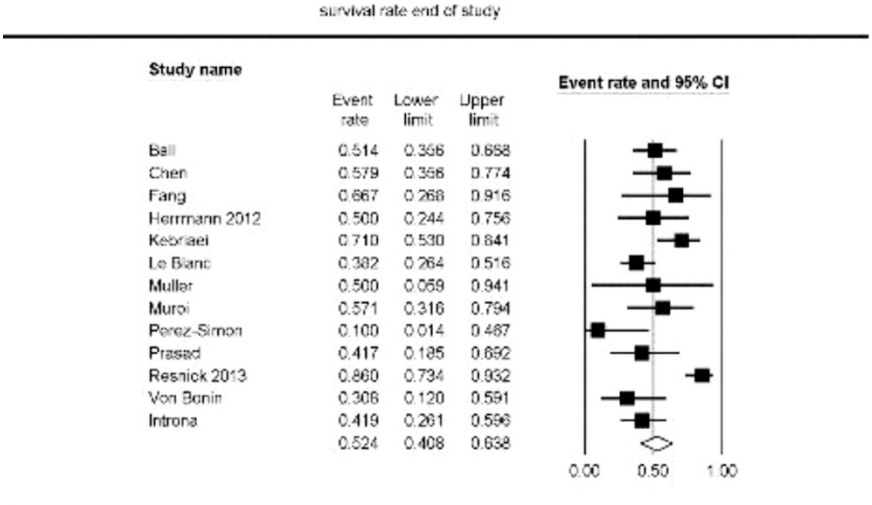
Introduction: Graft-versus-host-disease (GVHD) is the major limitation of allogeneic transplants. Due to the results of compelling phase I-II studies (including the EBMT trial) in acute GVHD, the use of mesenchymal stromal cells (MSC) has become standard practice in many countries even though a phase III trial in the United States (Prochymal) reported negative results. We conducted a meta-analysis of currently available published and unpublished data using the PRISMA Statement to formulate the reporting.

Materials (or patients) and methods: A literature search (1996-2013) included the Medline, EMBASE, Ovid & Cochrane CENTRAL databases. Outcomes measured were response rates (RR) & 6 month survival. Unpublished studies/conference abstracts from ASBMT, SIOP, EBMT, ASH were also searched. Quality Appraisal for the Risk of Bias was done via RoBANS since most of the studies were non-randomized. A random-effects model was used to pool outcomes across studies due to anticipated heterogeneity. Survival was assessed at 6 months and at the end of the study. Subgroup analyses included (a) pediatric vs adult (b) dosage of MSCs delivered (c) type of MSC lysate utilized and (d) source of MSCs.

[P396]



Results: Searches yielded 628 published & unpublished studies, 562 were excluded mainly due to usage of MSCs for non-GVHD conditions. Additional information on survival at 6 months was obtained for 13 studies from investigators which was included in the final cohort. Because of the absence of survival data from the Prochymal Study, this study was not included in the meta-analysis. For the survival meta-analysis, 292 patients were included. The median number of days from the onset of acute GVHD to MSC infusion varied between studies (2-46d). The MSC dose delivered was variable in different studies (0.3-9 x 10e6/kg). The 6 m survival was 0.63 (range 0.50-0.74; $I^2 = 41$); survival (end of study) was 0.52 (range 0.41-0.64; $I^2 = 67$). There was no significant difference in relative risks (RR) based on age, lysate type, dose of MSC delivered, and no significant correlation in survival between adult vs pediatric groups ($P = 0.43$), or between the dosage of MSCs ($P = 0.48$). MSC studies which had utilized Fetal Bovine Serum (FBS) lysate compared to platelet lysate for culture demonstrated a superior response (0.8 vs 0.62 respectively; $P = 0.02$). MSC responders had a 6 m survival of 0.63 (0.51-0.74);nonresponders had a 6 m survival of 0.16 (0.07-0.35), with no heterogeneity ($I^2 = 0$). ORR and CR were significantly associated with time from diagnosis with early administration of MSCs favoring better responses ($P = 0.03$ and $P = 0.02$ respectively). The 6 m survival from non-MSC studies which have been analyzed in recent ASBMT guidelines was compared with that of MSC studies.The average weighted event rate was found to be 0.42 from the onset of GVHD in non-MSC studies, compared to 0.63 with MSCs. Additional work is in progress to analyze the comparisons of survival from the onset of MSC and non-MSC treatments.
Conclusion: Totality of evidence from this meta-analysis indicates that MSCs are an acceptable treatment for acute steroid refractory GVHD. Randomized clinical trials are urgently needed for comparison of MSC vs non-MSC modalities.
Disclosure of Interest: None declared.

P397
Functional Evaluation of T-cells Generated from WT1-TCR Transduced Human Hematopoietic Stem Cells Using the OP9-DL1 Coculture System

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Introduction: Chemotherapy leads to cure of acute myeloid leukemia (AML) in less than half of the patients. Stem cell transplantation can be used as an immunotherapeutic treatment to cure the patient, but carries a high risk of toxicity and mortality. Moreover, not all patients have a suitable donor. We have developed a novel immunotherapeutic treatment, in which we generate in vitro, starting from hematopoietic precursor cells (HPC), T-cells that recognize WT1, a tumor antigen that is overexpressed on 70% of the AMLs.
Materials (or patients) and methods: $CD34^+$ cells isolated from cord blood and mobilized peripheral blood mononuclear cells were cultured on OP9-DL1 in the presence of the cytokines IL-7, Flt3-L and SCF, for 2 weeks, until T-cell commitment. Subsequently, they were transduced with a WT1-TCR (H. Stauss) or a CMV-TCR (M. Heemskerk), and again co-cultured until $CD4^+CD8^+$ double positive cells were abundantly present. At that point, the agonist peptide WT1 or CMV resp was added to the culture together with IL-7, and 5 days later cells were harvested and expanded, in the presence of IL-2, or IL-7 + IL-15.
T-cells were evaluated using a ^{51}Chromium release assay, for cytotoxicity against WT1 and HLA-A2 positive and negative targets. Also, upon activation, production of IFN-g was evaluated using ELISA.
Immunodeficient 6-8 weeks old NSG mice were irradiated (200 cGy), and 24 hours later injected intravenously with either a luciferase-positive, WT1, HLA-A2 transduced K562 cell line (R. Stripecke), or luciferase-transduced, HLA-A2 +, WT1 + primary AML cells, and 24 hours later, with $5x10^6$ or 10^7 WT1 + T-cells or CMV + T-cells (negative control). Mice were evaluated using the IVIS bioluminescence assay.
Results: We observed that a mix of WT1-TCR CD8 + and CD4 + T-cells (50%/50%) was generated if the cells were expanded after harvest from the coculture using the combination of the agonist peptide, IL-7 and IL-15. Using ^{51}Cr release assay and ELISA, we could show that upon activation, the T-cells showed specific cytokine production and efficient killing of tumor cells. We observed that the luciferase +, WT1, HLA-A2 transduced K562 cell line homed to ovaria and brain (female mice) or liver, testes and brain (male mice) when injected intravenously, and these are largely sanctuary sites, not reached by the T-cells, therefore resulting in low efficiency. When this cell line was injected subcutaneously in the hind flank, mice showed significant swelling of the resp limb and needed to be euthanized for ethical reasons before full evaluation was possible.
Currently, experiments are ongoing evaluating the efficacy of the WT1 T-cells against luciferase transduced primary AML cells (after short or long term expansion culture on MS-5), as these cells are expected to home to the bone marrow and blood of the mice, and therefore reflect more the physiological situation, and can be more easily reached by the T-cells. Results of these experiments will be presented at EBMT.
Conclusion: We have shown that, using the OP9-DL1 model, we are able to generate large numbers of high-avidity tumor-specific naïve and resting T-cells. After expansion and activation, these cells show specificity and functionality in vitro and are currently evaluated in an in vivo mouse model.
Disclosure of Interest: None declared.

P398
Decidual stromal cells induce IL-2 insensitivity in allo-stimulated T cells *in vitro*

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Introduction: Stromal cells have immunomodulatory features and are increasingly being used as cellular therapy for inflammatory conditions following allogeneic stem cell transplantation. Although they suppress alloresponses *in vitro*, these cells induce a high production of interleukin 2 (IL-2). The present study elucidates IL-2 production and responsiveness in allo-stimulated T cells cultivated with decidual stromal cells (DSCs).
Materials (or patients) and methods: Elisa was used to determine IL-2 concentrations in supernatants of mixed lymphocyte reactions (MLRs) co-cultured with or without DSCs (1:10 DSC/responder PBMC ratio). Flow cytometry was used to determine expression of the IL-2 R subunits and phosphorylated STAT5.
Results: IL-2 concentration in MLRs with DSCs was significantly increased compared to MLR controls (median 1.9 ng/ml and 0.02 ng/ml, respectively, $P = 0{,}0001$, $n = 14$). This was contact-dependent; DSCs added in transwells to MLRs did not increase IL-2 concentration. PBMCs co-cultured with DSCs did not significantly increase IL-2 concentration compared to PBMCs alone. In CD4 + T cells, expression of the IL-2 R alpha chain (CD25) was upregulated when DSCs were co-cultured with MLRs (median 1.9-fold increase, $n = 21$, $P = 0.0002$). However, the frequency of cells expressing the signaling

IL-2 R beta chain (CD122) was almost abolished when DSCs were added to MLRs (2.8% vs 15.8% in control MLR, $n = 17$, $P = 0.0009$). Additionally, intensity of CD122 expression was decreased 1.3-fold ($n = 11$, $P = 0.001$). The IL-2 R common gamma chain was abundantly expressed on CD4 + cells, although was significantly reduced by DSCs (83.6% vs 95.8%, 2.1-fold intensity decrease, $n = 17$, $P = 0.0003$ and $P < 0.0001$, respectively.) Depletion of CD122 could be reproduced when 3 ng/ml recombinant IL-2 were added to MLRs day three of incubation. Activation of T cells was determined by investigating STAT5 phosphorylation (pSTAT5). The intensity of pSTAT5 decreased 1.36-fold in CD4 + T cells when MLRs were co-cultured with DSCs ($n = 12$, $P < 0.002$). STAT5 phosphorylation in FOXP3 + regulatory T cells was close to 100% and was thereby not affected by DSCs ($n = 6$).

Conclusion: To conclude, these data suggest that DSCs induce a surge of IL-2 production that alter IL-2 R composition and reduce STAT5 activation capability in allo-stimulated T cells.

Disclosure of Interest: None declared.

P399

Preclinical Studies and GMP-conform Scale-up of CMV-specific Cytokine-induced Killer (CIK) Cells Targeting Leukemia and Infections

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Introduction: Human cytomegalovirus (CMV) infections are asymptomatic in most immunocompetent individuals but infection and reactivation after allogeneic hematopoietic stem cell transplantation (HSCT), especially in patients who received T cell depleted haploidentical stem cells, remains a major cause of morbidity besides relapse. Adoptive immunotherapy to prevent or treat impending relapse and in addition restore virus-specific cellular immunity, is clearly an attractive treatment option. Therefore we generated CMV_{pp65} antigen-pulsed CIK cells resulting in dual anti-leukemic and anti-viral cytotoxicity.

Materials (or patients) and methods: For the preclinical studies and GMP-conform scale up CIK_{pp65} cells are generated *ex vivo* from peripheral blood mononuclear cells (PBMC) of CMV-seropositive healthy donors with INFγ, IL-2, anti-CD3 antibody, IL-15 and CMV_{pp65} peptide pool. Phenotype of the cells is identified by 10-color flow cytometry panel and cell-mediated lysis is evaluated targeting peptide-loaded target cells, infected fibroblasts and leukemia cell lines. For the GMP-conform scale up, the whole manufacturing process (production and quality control) of the CIK_{pp65} cells was performed under clean room conditions.

Results: A single stimulation with CMV_{pp65} antigen expands CMV-specific $CD8^+$ cells within CIK cells up to 23% after 15 days of expansion without sacrificing anti-tumor activity ($n = 12$). Interestingly, CMV-reactivity can be found within T cell ($CD3^+CD56^-$) as well as T-NK cell ($CD3^+CD56^+$) compartment. The lysis of pp65 loaded cells by CIK_{pp65} cells was significant higher as compared to conventional CIK cells (effector to target cell ratio of 5:1, 39.9 ± 21.6% to 13.6 ± 10.6%, $P < 0.01$). CIK_{pp65} cells also induced high cytotoxicity in CMV-infected fibroblasts (up to 55%, 10:1 E:T ratio). The anti-leukemic effect was retained in CIK_{pp65} cells, revealing a mean cytotoxicity of 71.5%, 60.7% and 37.8% against leukemia cell line THP-1 and 55.0%, 50.0%, 20.5% against K562 in 40:1, 20:1 and 5:1 E:T ratio, respectively. In contrast, the reactivity against allogeneic mismatched PBMC remained low (18% lysis, 40:1 E:T ratio). Cytokine secretion (granzyme B, IFN-γ, MIP-1α, TNF-α, Fas-L, IP-10, IL-10, IL-6 and IL-4) data reflected the cytotoxic nature of the cells and indicated towards a mainly T_H1 cell type character. Interestingly, memory phenotype analysis revealed that not all CIK_{pp65} cells were fully differentiated and therefore still have the potential to get activated and may be accessible for restimulation *in vivo*.

Changeover in GMP-conform cell medium and closed culture system did not influence the peptide-pulsing and expansion capacity of CMV-specific $CD8^+$ cells and CIK_{pp65} cells in total.

Conclusion: In conclusion CIK_{pp65} cells can easily be generated from donor PBMC within two weeks with just a single stimulation of pp65 antigen and might represent advantage to conventional CIK cells. Our pre-clinical data demonstrate the concomitant cytotoxicity of generated cells against leukemia cells and CMV, besides low alloreactivity and limited risk to induce GvHD. These results suggest CIK_{pp65} cells as a promising candidate for clinical application even in an HLA-mismatched haploidentical setting to treat patients which have both an apparent risk of CMV reactivation and leukemic relapse after allogeneic stem cell transplantation.

Disclosure of Interest: None declared.

P400

Collagen foam membranes colonized by human mesenchymal stromal cells efficiently inhibit allogenic T lymphocyte proliferation in vitro

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Introduction: Human mesenchymal stromal cells (MSC) can be expanded in vitro from most vascularized tissues.These cells are able to differentiate in multiple lineages and to control various inflammatory processes, including steroid-resistant graft versus host disease that may occur after grafting patients with allogenic hematopoietic stem cells. MSC thus represent a great hope as a therapeutic tool for either tissue reconstruction or anti-inflammatory therapies. In general MSC are injected as single cell suspensions and tend to vanish from the injection site when injected locally, and are very difficult to localize after systemic infusion. We therefore investigated in vitro whether MSC immobilized on a biocompatible scaffold retained their ability to inhibit allogenic T lymphocyte activation.

Materials (or patients) and methods: MSC precursors were harvested from femoral head remains after informed consent of patients undertaking hip surgery, and amplified with platelet lysate (PL). Amplified MSC (1xE5 cells in 10 µl, passage 2 or 3) were seeded on 6 mm-diameter collagen foam membranes (CFM) (ultrafoam collagen hemostat, Bard Limited, UK) for 2 hours. The seeded CFM were transferred in new wells and cultured for 19 to 120 days in PL. Mean CFM surface was estimated using a square grid as reference. CFM were then either fixed for microscopic examination, disrupted with collagenase type II for cell count and phenotype determination by FACS, or cocultured with allogenic T lymphocytes stimulated with anti-CD3 and CD28 mAb immobilized on microbeads (A3-28) to determine their immunomodulatory potency.

Results: MSC-seeded CFM transiently released cells during the first week of culture. From day 9 on CFM decreased in shape, increased in opacity and exhibited a much smoother surface than unseeded CFM. After 65 days the mean surface of the seeded CFM was half of the value prior to seeding ($n = 9$, $P <$ E-4, t-test). In one case, the CFM totally dissolved and MSC

formed an adherent layer in the dish. Microscopic examination of CFM slides stained with Goldner's dye obtained after 19 days of culture showed that MSC were localized both within CFM and on its surface where they formed a well-organized layer that was multicellular in some areas. Collagenase digestion of the membranes released viable cells in numbers corresponding to 50% of the seeded value. These cells were CD45-, CD105+, CD54+, CD73+, and CD106 and HLA-DRlow. MSC-seeded CFM prevented A3-28-induced allogenic T lymphocyte proliferation. T cell inhibition was effective even after 3 consecutive incubations of membranes with 3 different T lymphocyte preparations, whereas empty membranes never inhibited T lymphocyte proliferation.
Conclusion: These data show that MSC efficiently colonize CFMs, are long-lived in these structures and express similar markers than these observed in 2D-culture. Moreover MSC residing in CFM maintain their in vitro immunomodulatory properties, suggesting that MSC-loaded CFM could be used in vivo to control local inflammation. However before undertaking any clinical application several points require answers such as how cell colonization progresses in the membrane, whether MSC develop a proteolytic activity that could explain membrane shrinkage, and what is the cell cycle and differentiation status of cells in regard to their localization within the membrane. These issues are presently under investigation.
Disclosure of Interest: None declared.

Regenerative medicine

P401
Succesful treatment of psoriasis with allogeneic HSCT in a child with MDS, hypogammaglobulinemia, and monosomy 7

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Introduction: Hematopoietic stem cell treatment (HSCT) has been performed in treatment of some immune mediated disorders. However, there is no report, especially in children, that shows effect of HSCT on psoriasis.
Materials (or patients) and methods: A 3.5-year old girl diagnosed with psoriasis, hypogammaglobulinemia and pancytopenia in a local hospital at the age of 2 years was referred to our hospital after detection of 12% blasts and monosomy 7 in a bone marrow (BM) specimen. Physical examination revealed normal growth and diffuse psoriatic lesions throughout the skin. Laboratory investigations revealed hemoglobin 8.5 g/dL, mean corpuscular volume 92 fL, reticulocyte count 0.4%, leucocyte count $3.7x10^9$/L (absolute neutrophil count $0.4x10^9$/L), and platelet count $28x10^9$/L with 2% blasts on peripheral smear. Lymphocyte subset analysis revealed very low number of CD19+ cells (1%). T-lymphocyte stimulation by phytohemagglutinin disclosed normal results. Virologic, microbiologic and immunologic studies, DEB test, ferritin, vitamin B12 and folic acid levels were all normal. Bone marrow aspiration revealed 15% blasts and BM biopsy revealed 10-15% $CD34^+$ blastic cells and trilineage dysplasia. A clonal population with monosomy 7 was detected in 70% of metaphases studied. She was diagnosed with myelodysplastic syndrome (MDS), refractory anemia with excess of blast (RAEB). Due to progression of MDS to AML (23% blasts in BM) within 3 months, she was given AML-BFM induction treatment, and then underwent HSCT from her full-matched uncle. She achieved full engraftment without complication. Interestingly, her psoriatic lesions disappeared after HSCT and she is free of psoriasis for the last 1 year after BMT.
Results: Current hypothesis claims that in genetically predisposed persons, nonspecific stimulation of T-cells amplifies epidermal growth in psoriasis. Although number, distribution and activity of T cells were normal in our patient, disappearance of psoriatic lesions after BMT may indicate an intrinsic T-cell defect that is also a new finding. Exciting outcome of BMT in our patient may also indicate effect of allogeneic mesenchymal stem cells in the healing of psoriasis. A recent study disclosed aberrant proliferative activity, increased apoptosis rate, and different gene expression profile in bone marrow mesenchymal stem cells obtained from psoriatic patients, that lead to defective immune response.
Conclusion: Disappearance of psoriatic lesions in our patient may be explained by the immunomodulatory effect of allogeneic bone marrow mesenchymal stem cells or repair of T-cell defect.
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Disclosure of Interest: None declared.

Stem cell mobilization and graft engineering

P402
Real World Stem Cell Mobilisation Strategies in Patients With Multiple Myeloma Or Lymphoma In The UK

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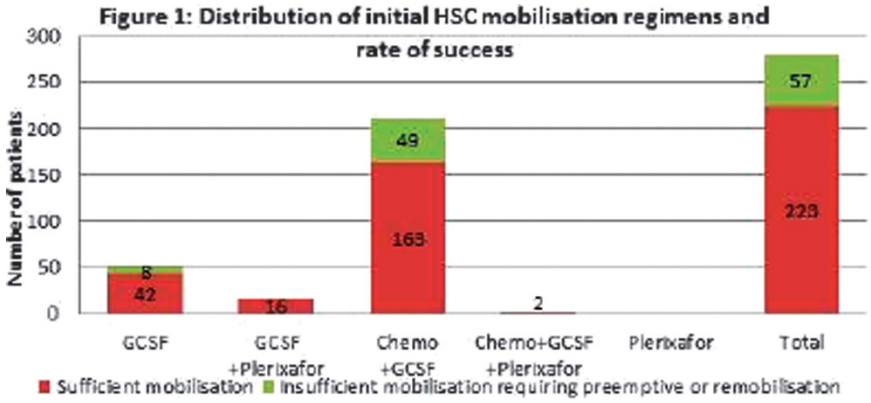
Introduction: Reported success rates of stem cell mobilisation prior to autologous haematopoietic stem cell transplant (HSCT) vary with little data available regarding strategies employed in institutional practice. This ongoing Sanofi-funded study (MOBILITY) aims to describe stem cell mobilisation pathways and outcomes in UK patients with lymphoma and multiple myeloma.
Materials (or patients) and methods: An observational research study conducted in 5 centres in England, Scotland and Wales with data captured retrospectively by the direct care teams from the medical records of patients with lymphoma or multiple myeloma, from start of mobilisation treatment (01/01/2013-31/12/2013) to day-100 post HSCT. Data will be collected for about 400 patients in total; we present here interim results (data cut-off 27/10/2014), with full results due in early 2015.
Results: This interim analysis includes 280 patients from 5 centres (range 19-82 patients/centre). 117 (63%) were male, mean (standard deviation, SD) age at start of mobilisation treatment was 56.7 (10.6) years. 150 (54%) patients had multiple myeloma and 130 (46%) lymphoma. At HSC mobilisation, patients had a mean (SD) 1.7 (0.9) previous lines of chemotherapy and 49 (18%) patients had had prior radiotherapy.

[P402]



Mobilisation was successful (i.e. patient proceeded to apheresis) in 223 (80%) patients following initial mobilisation regimen only. Distribution of treatments used and success rates are shown in Figure 1.

Pre-emptive treatment (increased dose of initial mobilisation agent(s) ± plerixafor) attempts were employed for 30 (11%) patients; 14 (47%) plerixafor + ongoing GCSF at an increased dose, 11 (37%) plerixafor + ongoing GCSF at same dose and 5 (17%) ongoing GCSF at increased dose. Delayed remobilisation was employed for 30 (11%) patients (2/30 (7%) following prior pre-emptive treatment); 14 (47%) patients received plerixafor + GCSF, 11 (37%) GSCF alone, 4 (13%) chemotherapy + GCSF and 1 (3%) chemotherapy + GCSF + plerixafor. Mean (SD) time from end of initial mobilisation to remobilisation ($n = 30$) was 6.8 (4.8) weeks.

Overall 274 (98%) patients were successfully mobilised and underwent apheresis; mean (SD) cell harvest was $6.4x10^6$ ($5.0x10^6$) CD34 + cells/kg over 1.7 (0.9) harvests. A yield of $>2x10^6$ CD34 + cells/kg was achieved for 262/274 (96%) patients. 227/274 (83%) patients who were successfully mobilised proceeded to autologous HSCT; median (range) time to neutrophil engraftment ($>0.5x10^9$/l) and platelet ($>20x10^9$/l) recovery was 11 (8-22) and 12 (6-38) days, respectively. Most common reasons not to proceed to HSCT were disease progression and patient not fit to proceed. 218/230 (95%) patients with data available were alive at day 100 post-HSCT.

Conclusion: In this multicentre study, patients were predominantly mobilised using Chemotherapy + GCSF. The failure rate of initial mobilisation attempts is similar to rates previously reported[1]. There appears to be a lack of consensus of optimal strategy as pre-emptive and remobilisation strategies were utilised in similar number of cases, however, mobilisation was ultimately successful in almost all cases. The high overall rate of successful mobilisation maximises the number of candidates who can be considered for autologous HSCT.

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Disclosure of Interest: A. Bloor Funding from: Received financial support from Sanofi to attend a conference, K. Wilson Funding from: Received honoraria and travel grants from Sanofi, N. Russell Funding from: Previously received speakers fees from Sanofi, A. Peniket: None declared, R. Dew Funding from: Under commission by Sanofi to design study, facilitate conduction of the study and scientific editorial services, T. T. Lin Employee of: Employee of Sanofi, K. Douglas Funding from: Received honoraria from Sanofi and Genzyme between 2009 and 2014 for speaker work and Medical Advisory Board participation in connection with the use of plerixafor within its licensed indications.

P403

Effect of transplanted CD26-positive cells for lymphocytes' reconstitution after autologous hematopoietic stem cells transplantation in patients with multiple myeloma – one center prospective study

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Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) remains the treatment of choice in multiple myeloma (MM) patients (pts). In earlier research it has been suggested that the expression of dipeptidyl peptidase-4 (DPP4, CD26) influences both the homing and lymphocyte reconstitution after AHSCT in pts with lymphoproliferative neoplasms. The aim of the study is to investigate the effect of transplanted CD26 + cells of the hematopoietic recovery and lymphocyte reconstitution in MM pts after AHSCT.

Materials (or patients) and methods: Forty eight pts with MM with median age 56 were undergoing AHSCT in our center in years 2011-13. Conditioning regimen was Melphalan 200. Number of all CD26 + cells, CD26 + lymphocytes, CD26 + monocytes and CD26 + and CD34 + cells were measured in harvested material. Number of lymphocyte's subpopulations (all lymphocytes CD3 +, helpers CD3 + CD4 +, suppressors CD3 + CD8 +, natural killer (NK) CD3-CD16 + CD56 +, cytotoxic NK CD3 + CD16 + CD56 +, lymphocytes B CD3-CD19 +) were measured in peripheral blood during regeneration period after AHSCT. In both flow cytometry was used. The hematopoietic regeneration was measured as following: the day of white blood cells' regeneration when WBC count reached $>1,0x10^9$/L, the day of granulocytes' regeneration when ANC $>0,5x10^9$/L and the day of platelets' regeneration when PLT $>20x10^9$/L.

Results: All pts successfully engrafted. The results of AHSCT are shown in table nr 1. As regards regeneration of hematopoietic cells after AHSCT it was shown that a higher number of transplanted CD26 + monocytes improves the reconstitution of suppressor ($P = 0,019$) and NK lymphocytes ($P = 0,0237$). A higher number of all transplanted CD26 + lymphocytes has a positive impact of the reconstitution of suppressor lymphocytes ($P = 0,0054$), whereas a higher number of all transplanted the CD26 + cells improves the regeneration of cytotoxic NK ($P = 0,0126$) and helper lymphocytes ($P = 0,0261$). There were no confirmed adverse effects of the number of CD26 + cells on the hematopoietic regeneration0 and lymphocytes B reconstitution after AHSCT.

Conclusion:

1. Our research shows that the number of transplanted CD26 + cells may improve immune reconstitution after AHSCT

[P403]

Parameter	Median	Range	Mean	Standard deviation
Number of transplanted WBCx10^8/kg b.w.	4,26	0,73-18,8	5,43	4,36
Number of transplanted CD34+cells x10^6/kg b.w.	3,36	2,2-8,2	3,52	1,28
Number of transplanted CD26+ lymphocytes [10^9/L]	46,5	9-148	53,6	30,8
Number of transplanted CD26+ monocytes [10^9/L]	3,65	0-82	8,03	13,05
Number of all transplanted CD26+ cells [10^9/L]	50,42	9,6-213	62,5	23,2
Regeneration				
WBC >1x10^9/L (day)	13	10-20	13	2,64
ANC >0.5x10^9/L (day)	13	9-20	13,3	2,16
PLT >20x10^9/L (day)	14	11-20	14,1	2,18

Table 1. The number of transplanted cells and regeneration during the procedure AHSCT in pts with MM

in patients with multiple myeloma, which was not clearly demonstrated before[1,2,3]. As is well known faster lymphocyte reconstitution after AHSCT is associated with improved patient survival[4]. Therefore, the greater the number of transplanted autologous CD26+ cells may be associated with improved survival, which, however, needs further investigation.

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Disclosure of Interest: None declared.

P404

Age and Body Mass Index are major determinants of hematopoietic stem cell mobilization in related donors

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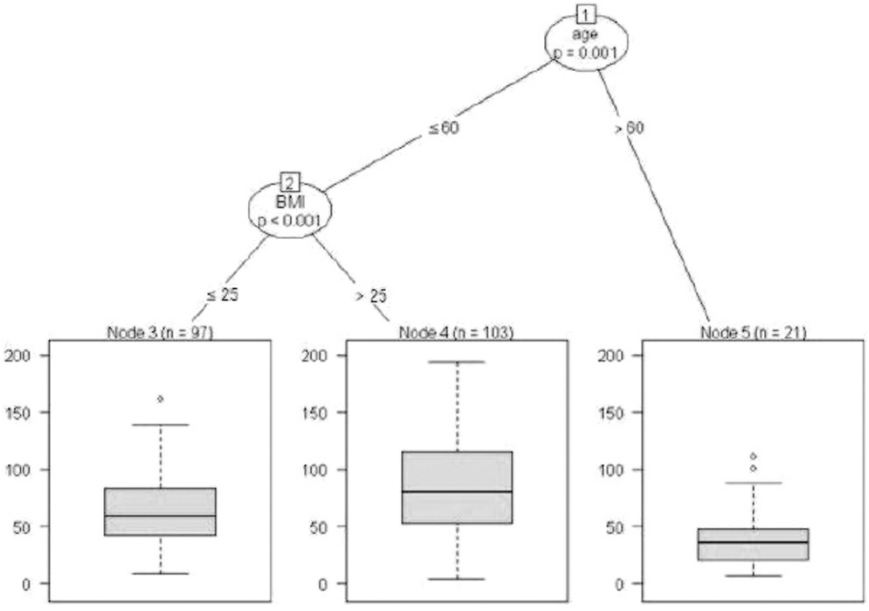
Introduction: Changes in allogeneic hematopoietic stem cell transplantation practices also impact on donors search procedures: the growing proportion of haplo-identical transplants makes especially necessary to develop criteria for donor selection beyond strict HLA-identity, since multiple related donors are commonly identified. While several factors are known to affect peripheral blood stem cell mobilization in healthy donors, validation of robust and easy-to-use predictive criteria for donor selection, such as sex, age or body weight, remains necessary. In order to better define the physiological profile of good mobilizer, we retrospectively analyzed 331 related donors mobilized at a single institution.

Materials (or patients) and methods: Between January 2008 and August 2014, 331 unique related donors were mobilized and consecutively harvested, 25% in the haplo-identical setting ($n = 83$) and 75% in the geno-identical setting ($n = 248$). Donor population was 44% female ($n = 146/331$)

[P404]



with age ranging from 18 to 77 years (median 50) and median weight of 73 kg [44-125]. All donors received 10 μg/kg rhu-G-CSF (median 10.7 [6.4-16.3]) from day 1 to day 5; on day 5 circulating CD34 cells were evaluated using single platform technology (Stem Cell Enumeration kit, Becton Dickinson). Median circulating CD34+ cell number on day 5 was 64/μl [4-206].

Results: Univariate logistic regression shows that sex, age, weight, height, Body Mass Index (BMI) and G-CSF dose per kg were statistically associated with the number of circulating CD34+ cells. On multivariate analysis, BMI was statistically the most significant factor ($P = 1.8e-9$), followed by age ($P = 1.5e-8$), dose of G-CSF/kg ($P = 7.2e-6$) and sex ($P = 0.02$). In order to facilitate interpretation of these data, we transposed these results into a decision tree, using the R software and two sets of donors (learning set, $n = 221$; validation set, $n = 110$). This statistical analysis leads to distinguish three distinct groups of individuals (Figure 1), with age being the first cut off and BMI the second. Briefly, donors older than 60 years are the worst mobilizers (median CD34/μl = 41); younger donors are good mobilizer when BMI is > 25 (median CD34/μl = 87) while BMI ≤ 25 is associated with intermediate circulating CD34+ cell counts (median CD34/μl = 65).

Conclusion: Our retrospective analysis of a large cohort of related donors allows us to propose a clinically-meaningful decision tree that can be routinely used as additional criteria to define the most appropriate donors with regards to CD34 mobilization. Use of this decision tree should be integrated with other variables such as HLA identity, sex, CMV status, ABO group, etc. Our analysis also outlines the known role of BMI in hematopoietic stem cell mobilization, and raises the issue of the relationship between the adipose tissue and the biological response to rhG-CSF.

Disclosure of Interest: None declared.

P405

Autologous haematopoietic stem cell mobilization in HIV positive patients: the role of plerixafor in association with G-CSF and chemotherapy

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Introduction: High-dose chemotherapy and autologous stem cell transplantation is a crucial treatment option for hematologic malignancy patients. Moreover, the incidence of Hodgkin disease (HD) and non-Hodgkin lymphoma (NHL) in HIV-infected individuals is much greater than in the HIV-negative population. The advent of highly active antiretroviral therapy (HAART) altered the natural history of HIV infection and allowed to use ASCT as a safe and successful therapeutic option in patients with HIV-related lymphoma, as it has been reported as a useful procedure. As current mobilization regimes often do not provide adequate numbers of CD34+ cells, Plerixafor, a CXCR4 antagonist, has been approved for use in combination with G-CSF. As HIV positive patients are frequently poor mobilizers, we administrated Plerixafor on demand in our mobilization procedures.

Materials (or patients) and methods: This is a retrospective, registry-based analysis. Five HIV positive patients, four of them affected with non Hodgkin Lymphoma and one with Hodgkin Lymphoma, received G-CSF plus chemotherapy; all patients received 1 dose of plerixafor on demand. Plerixafor was administrated at the dose of 0.24 mg/kg 6-8 hours before apheresis.

Results: The addition of plerixafor to G-CSF and chemotherapy resulted in a twofold increase in peripheral blood CD34+ cell count. One patient failed to achieve the CD34+ peak of more than 20 cells/ml and did not undergo apheresis. Four patients met the target of 6 × 10(6) CD34+ cells/kg, three of them with 2 days apheresis, one with a single collection procedure. Two of them underwent stem cell transplantation, and neutrophils and platelets engraftment were on expected time (11 days and 12 days respectively for the first patient, 10 days and 10 days respectively for the second patient). Plerixafor-related toxicities were minimal. Engraftment kinetics, graft durability and transplant outcomes demonstrated no unexpected outcomes.

Conclusion: Our study showed that, in our experience, Plerixafor has the same efficacy and safety as other published studies. Moreover, preliminary data show that plerixafor is usefull also in HIV positive patients, in which a decrease number of apheresis procedures can be related to a increase safety for both patients and operators. Considering the cost, the use of Plerixafor in such patients may require careful evaluation in order to offer ASCT opportunity to HIV positive patients. HIV infection alone should not preclude an attempt to obtain stem cells in candidates for autologous transplant as the results are comparable to the HIV-negative population.

Disclosure of Interest: None declared.

P406

Chemomobilization strategies with intermediate-dose etoposide and delayed use of G-CSF for autologous stem cell transplantation in patients with hematologic malignancies: high effectiveness and delayed platelet recovery

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Introduction: High dose cyclophosphamide with G-CSF is one of the most widely used mobilization strategy but a proportion of patients fail to collect the minimum number of cells required. So, effective and safe alternative mobilization approaches is needed.

Materials (or patients) and methods: We compared the efficacy of chemomobilization with intermediate-dose etoposide and delayed addition of G-CSF versus high dose cyclophosphamide plus G-CSF versus G-CSF alone. We analyzed total 111 patients who received autologous stem cell transplantation in Korea university medical center (55 multiple myeloma, 49 lymphoma, 7 Acute myeloid leukemia). Thirty six patients received intermediate-dose etoposide (375 mg/m^2/day) on day 1,2 and 49 patients received high dose cyclophosphamide (3 g/m^2/day) on day 1. Filgrastim (10 mcg/kg/day) was injected from 10 days after the completion of chemomobilization until the last day of apheresis in both group. Twenty six patients received only filgrastim (10 mcg/kg/day) with same administration schedule.

Results: Median CD34 positive cell yield was significantly higher in etoposide group (10.71×10^6/kg) than cyclophosphamide group (6.49×10^6/kg) and G-CSF group (4.0×10^6/kg) ($p < .001$). The rate of successful mobilization ($\geq 5 \times 10^6$/kg) was also significantly higher in etoposide group (83.3%) than cyclophosphamide group (52.1%) and G-CSF group (23.1%) ($p < .001$). In etoposide group, only 1 patient experienced mobilization failure ($CD34^+$ cell yield $< 2.0 \times 10^6$/kg). The required number of apheresis to achieve the minimal $CD34^+$ cell dose of 2×10^6/kg was fewer in the etoposide group than other groups (median, 1 versus 2, $P = 0.027$). There were no significant differences in neutrophil ($P = 0.563$) and platelet count ($P = 0.22$) at nadir between etoposide and cyclophosphamide group. Severe febrile neutropenia and treatment related mortality were not observed in any group. Days of neutrophil recovery to 0.5 x 10^9/L were not different between three mobilization groups. However, platelet recovery to 20 x 10^9/L without transfusion was achieved at a median 17 (9-55) in etoposide group, 11 (8-41) in cyclophosphamide group and 14 (8-55) in G-CSF group ($P = 0.021$). And number of platelet transfusion was also higher

in etoposide group ($P = 0.035$). Three patients in the etoposide group experienced engraft failure although they received more than 2×10^6/kg CD34$^+$ cells.

Outcomes	*Etoposide (36)*	*Cytoxan (49)*	*G-CSF (26)*	P *value*
Total CD 34$^+$ cell count ($\times 10^6$/kg)	10.71 (1.32-51.1)	6.49 (0.12-18.7)	4.0 (0.34-14.31)	$<.001$
Total CD 34$^+$ cell $\geq 5 \times 10^6$/kg	30 (83.3)	25 (52.1)	6 (23.1)	$<.001$
Mobilization failure $<2 \times 10^6$/kg	1 (2.8)	12 (24.5)	7 (26.9)	0.009
Number of apheresis to achieve the				
minimal CD 34$^+$ yield ($\geq 2 \times 10^6$/kg)	1 (1-4)	2 (1-7)	2 (1-5)	0.027
Febrile neutropenia (%)	9 (25)	17 (34.7)		0.338
Day to neutrophil engraftment	11 (9-14)	11 (10-25)	12 (10-25)	0.13
Day to platelet engraftment	17 (9-55)	11 (8-41)	14 (8-55)	0.021
Number of platelet transfusion	7 (1-98)	3 (1-27)	4 (1-35)	0.035

Conclusion: Combination strategy with intermediate-dose etoposide and delayed administration of filgrastim is highly effective and safe mobililzation method. But further studies will be needed to determine the effect of delayed platelet recovery.
Disclosure of Interest: None declared.

P407
Plerixafor in healthy allogeneic donors in cases of inefficient G-CSF mobilization

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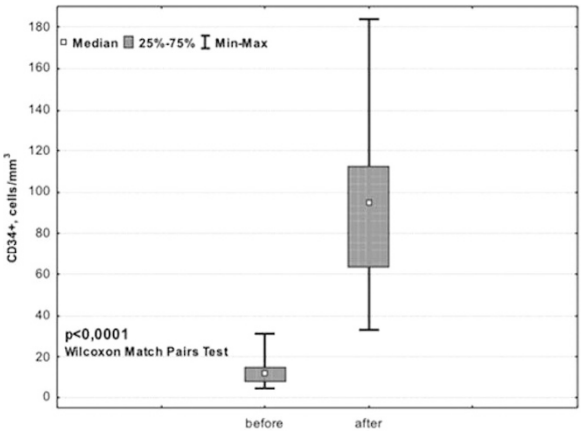
Introduction: Optimal dose of CD34 + cells is one of determinants for success of hematopoietic stem cell transplantation (HSCT). We studied effect of Plerixafor in healthy allogeneic donors not able to achieve optimal CD34 + cell counts in peripheral blood after G-CSF stimulation for harvest of sufficient number of stem cells for transplantation with.
Materials (or patients) and methods: According to the Institutional protocol of hematopoietic stem cells mobilization, all allogeneic donors received G-CSF (8-12 μg/kg) s.c., during 5 days. CD34 + cells count in peripheral blood was measured on the 4th day of mobilization. From January 2009 to June 2014 all poor mobilizers (donors with less than 20 CD34 + cells/μl on 4th day of G-SCF stimulation) received G-CSF and additional stimulation with Plerixafor, 0,24 μg/kg bw subcutaneously 12 hours prior to apheresis with subsequent measurement of CD34 + cells on the day of apheresis.
Results: During study period we used Plerixafor in 17 (18,8%) from 90 healthy donors: 15 of them due to inability to achieve target CD34 + cells count (20 cells/μl) on 4th day of G-SCF stimulation and 2 donors due to necessity achieve greater collection efficacy. After 4th day of G-CSF median number of CD34 + cells in peripheral blood was 12,5 cells/μl (4,7-31,2 cells/μl).
According to the protocol, Plerixafor was administered subcutaneously 12 hours prior apheresis (dose -0,24 μg/kg – 15 donors; 0,12 μg/kg – 2 donors). At the time of apheresis, CD34 + cells count in peripheral blood of all healthy donors dramatically increased to 33,2–183,7 cells/μl (median -95,1 cells/μl), and absolute CD34 + cells count in apheresis product was 259×10^6–770×10^6 cells (median - 475,0 x10^6 cells). Two donors received additional stimulation with Plerixafor in the same dose before second apheresis. Amount of CD34 + cells prior to second apheresis slightly increased from 63,1 to 71,6 cells/μl in one donor, and decreased from 183,7 to 42,6 cells/μl.



Mild toxicity of Plerixafor was noted in 6 of 17 healthy donors.
Conclusion: Our results are convincing enough to draw conclusions not only about efficacy, but also on the appropriateness of Plerixafor mobilization as graft's "salvation" in the cases of inefficient G-CSF mobilization.
Disclosure of Interest: None declared.

P408
Haploidentical hematopoietic stem cell transplantation in pediatric patients: comparison of depletion efficacy and engraftment according to in vitro T cell depletion method

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Introduction: We compared the efficacy of CD3 depletion with TCRαβ depletion in haploidentical stem cell transplantation (HHCT) for children and adolescents with malignant or non-malignant diseases.
Materials (or patients) and methods: Sixty-seven HHCTs were performed in 57 patients (SAA 19, MDS 7, AML 17, ALL 7, NBL 1, NHL 2, CDA 1, Rhabdomyosarcoma 1, Ewing's sarcoma 1, WAS 1) using CD3-depleted (HHCT-CD3, $n = 38$) or TCRαβ-depleted (HHCT-TCRαβ, $n = 29$) grafts from haploidentical family donors between 2008 and 2014 at AMCCH. A total of 67 graft manipulations were done with anti-CD3 ($n = 38$) or anti-TCRαβ ($n = 29$) microbeads (CliniMACS, MiltenyiBiotec). Donors included mother ($n = 36$), sibling ($n = 18$) and father ($n = 13$). We sought toobtain at least 4x10^6 CD34$^+$ cells/kg of recipient. MMF and cyclosporine or FK506 were used for GVHD prophylaxis.
Results: As for 67 graft manipulations, mean recovery of CD34$^+$ stem cells after CD3 depletion and TCRαβ depletion were 82.4% and 89.8% ($P = 0.04$), respectively. Mean depletion efficacy of CD3$^+$ Tcells after CD3 depletion was 3.1 log and that of αβ$^+$ Tcells after TCRαβ depletion was 3.6 log ($P = 0.01$). Of a total of 67 HHCTs, 3 (4.5%) experienced primary graft failure(GF) and additional 6 (8.9%) experienced graft rejection(GR) after HHCT-CD3. All 9 GF/GR were rescued with a second HHCT. There were neither GF nor GR in HHCT-TCRαβ. The median day of neutrophil engraftment was 10 days (range, 9-15) post-transplant which was not different according to depletion methods ($P > 0.05$). However, platelet engraftment was faster in HHCT-TCRαβ compared to HHCT-CD3 (median 16 days; 17 days for HHCT-TCRαβ vs 31 days for HHCT-CD3, $P = 0.03$).
Conclusion: TCRαβ depletion is a highly effective method to deplete T cells along with enrichment of CD34$^+$ stem cells. Given the high engraftment rate, HHCT using TCRαβ depletion is a promising approach in children and adolescents who lack a suitable donor.
Disclosure of Interest: None declared.

P409

"Plerixafor on-demand embedded with chemotherapy and G-CSF" as first line mobilization strategy in Lymphoma and Multiple Myeloma reduces rate of CD34+ mobilization failure with limited increase of costs. A GITMO study

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Introduction: For patients who mobilize poorly, the combination of Plerixafor (PLX)+ Granulocyte Colony-Stimulating Factor (G-CSF), as second mobilization attempt, is effective in 65-75% of cases. However, repetition of a second mobilization course delays time for transplantation and increases workload in harvest centers. We have previously developed an algorithm for the use of PLX "on demand" in association with chemotherapy and with G-CSF (Milone, Transfusion 2013). Thus, we designed a multicenter prospective trial to establish value of "PLX on-demand" embedded into these chemotherapy schedules as first line mobilization in MM and LYM patients. Herein, we report the final analysis of this study (registered as Study 35/VE/2012).

Materials (or patients) and methods: Primary end point was the reduction in failure of CD34+ cells mobilization in PB and the study was powered to detect a 50% decrease in mobilization failure, namely from 14% to 7%, this requires a sample size of 207 evaluable patients. Inclusion criteria were: diagnosis of MM or LYM (HD and NHL), age 18-70, first attempt in mobilization of CD34+ cells using HD-CTX (4 gr/m2) or DHAP plus G-CSF at dose of 5-10 mcg/m^2. PLX was planned on demand if, on day +13/+15 after chemotherapy, CD34+ cell count in PB was below 20x10^6/l, or if first apheresis yielded a CD34+ $<$1x10^6/kg. Results were analyzed according to "Intention to PLX Treatment". From April 2012 to November 2014, 215 patients were enrolled. In four cases, mobilization was interrupted. Thus, 211 patients were analyzed according to "Intention to PLX Treatment", mean patient's age was of 53 y. (18-73), 120 were male (56%) and 91 female (44%), 130 patients (61%) were affected with MM and 81 (39%) with Lymphoma, 141 patients (66%) received as mobilization chemotherapy HD-CTX (4 gr/m^2) and 70 patients (33%) received DHAP.

Results: Overall success rate on CD34+ mobilization (defined as a CD34+ count in PB $>$ 20x10^6/l) was 96.4%. Mobilization failure was registered in only 3.0% of MM and in 6.9% of Lymphoma. Overall success rate in harvesting a CD34+ $>$2 x10^6/kg was 95.4% (MM: 97.0,%; Lymphoma: 87.7%). 182/183 patients (99%) were correctly predicted to reach a successful mobilization without requiring PLX. Twenty-seven patients were predicted to not reach successful mobilization, 18 of them received PLX and 15/18 (83%) had a successful mobilization. Overall the rate of PLX indication according to the algorithm was only in 12.8% of all patients (MM 10%, Lymphoma 17%) thus increase of cost is limited.

Conclusion: Compared to chemotherapy+G-CSF, "On demand use of PLX in association with chemotherapy and G-CSF" is able to halve the mobilization and harvest failure rate: failure to reach the minimun harvest (CD34+: 2x10e6/Kg) in MM patients is reduced from 10% to 3.0% and in Lymphoma patients from 25-30% to 12.4%. Since the high specificity of our algorithm in identifying poor mobilizer patients, these results can be reached with limited use of PLX. Thus, our on demand strategy has, compared to other way of using PLX, a very favorable cost/efficacy ratio.

Disclosure of Interest: G. Milone Funding from: none, Employee of: none, Personal Interest: none, M. Martino Funding from: none, Employee of: none, Personal Interest: none, Conflict with: none, A. Di Marco: None declared, S. Leotta: None declared, A. Cupri: None declared, A. Spadaro: None declared, P. Scalzulli: None declared, A. Romano: None declared, D. Berritta: None declared, M. Parisi: None declared, A. Olivieri: None declared, F. Ciceri Funding from: none, Employee of: none, Personal Interest: none, Conflict with: none, G. Tripepi: None declared.

P410

Use of Biosimilar G-CSF for Hematopoietic Stem Cell Mobilization in Healthy Unrelated Donors: Interim Results of a 10-Year Follow-up Study

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Introduction: Some professional bodies have debated over the routine use of biosimilar G-CSF in healthy stem cell donors because of the paucity of safety data in this setting. In agreement with European regulators, a 10-year post-marketing study is being conducted to assess efficacy and long-term safety of biosimilar G-CSF (Zarzio®, Sandoz) in stem cell donors.

Materials (or patients) and methods: This is a prospective, non-interventional two study with a planned minimum of 200 donors from the German Stem Cell Donor registry. Donor selection, evaluation, treatment, collection and follow-up are performed according to standard procedures. Mobilization with standard-dose biosimilar G-CSF is followed by one or two large-volume aphereses, depending on mobilization efficiency and required stem cell dose. Data from the first pre-specified interim analysis are presented as median (range).

Results: Between May 2011 and this interim analysis, a total of 121 donors had been recruited, of whom 119 had completed mobilization and apheresis. All donors were Caucasian, 26% were female. Their age was 35 (19-58) years, BMI was 26 (19-42) kg/m^2. Duration of G-CSF exposure was 1 ($<$1%, apheresis cancelled on day 1 of stimulation), 9 (90%) or 11 twice-daily doses (9%) at a dose per injection of 4.3 (2.9-5.4) μg/kg/BW. Stem cell mobilization was effective in all donors, with an increase of circulating CD34+ cells to 100 (34-284)/μL. Maximum allowed apheresis duration was 300 minutes per session on up to 2 successive days. In the interest of donor safety and comfort, second-day aphereses were only performed if after the first apheresis $<$5x10^6/kg CD34+ cells were collected. A dose of 4.5x10^6/kg CD34+ cells was considered sufficient based on published data for stem cell dose-related transplantation outcomes. In our cohort, 8.8 (4.5-25.5) x10^6 CD34+ cells/kg were collected after one (91%) or two (9%) aphereses, thus providing a sufficient stem cell dose for all recipients. Acute adverse events (AEs) were frequent but well tolerated and consistent with known toxicities of G-CSF. The most frequent were bone pain (86%) and headache (27%) during mobilization. Lymphocyte and neutrophil count, lactate dehydrogenase, alkaline phosphatase, aspartate aminotransferase and uric acid were all elevated at the time of apheresis. Three probably / possibly drug-related serious AEs were reported (chest pain, chest pain plus dyspnea and benign thyroid neoplasm), all of which resolved. At time of interim analysis, 85 (74% eligible donor recall rate), 65 (69%) and 51 (62%) donors had completed 1, 6 and 12 months follow-up, respectively. All laboratory values had normalized by the 1-month follow-up visit, including leukocyte and platelet counts. Outcome data from day-100 reports were available for 57% of recipients. All patients were engrafted with neutrophils

(16 (7-43) days) and platelets (14 (6-56) days); aGvHD was reported for 57% (any GvHD), 34% ($\geq$°2) and 13% ($\geq$°3).
Conclusion: Interim results from this ongoing study indicate that Zarzio® is effective for allogeneic stem cell mobilization with an acute-phase safety profile in line with the known toxicities of G-CSF; stem cell products were clinically effective. To date, AEs observed are as expected and no safety signals have been observed that would question the use of Zarzio® for healthy donor stem cell mobilization.
Disclosure of Interest: P. Becker: None declared, S. Brauninger: None declared, H. Bialleck: None declared, B. Luxembourg: None declared, M. Schulz: None declared, M. Wiesneth: None declared, P. Reinhardt: None declared, J. Mytilineos: None declared, C. Seidl: None declared, A. Schwebig Employee of: Sandoz Biopharmaceuticals/Hexal AG, A. Kolpakova Employee of: Sandoz Biopharmaceuticals/Hexal AG, H. Schrezenmeier: None declared, E. Seifried: None declared, H. Bönig Funding from: Hexal AG and Chugai Pharmaceuticals

P411
A refined composite clinical score for the early identification of the predicted Poor Mobilizers (PM): a GITMO analysis

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Introduction: A GITMO panel recently proposed criteria to define proven and predicted Poor Mobilizer (PM) in MM and Lymphoma patients (pts) (Olivieri A. et al BMT 2011). We made a retrospective survey in 17 GITMO centers, including 1318 consecutive mobilization procedures, from 2009 to 2013, to validate these criteria.
Materials (or patients) and methods: As an interim model did not allow a satisfactory predictive model, a composite score, based on the same GITMO criteria, plus the pre-mobilized platelet count (Lanza et al, Transfusion 2013) has been tested in a training set of 592 pts: 351M, 241 F; 252MM, 255NHL and 85HD. Median age was 55yrs (range 15-76); 59% had BM involvement before mobilization (extensive in 37%:$>$30%). 59 (10%) had a previous mobilization failure.
Results: 34/592 pts (6%) did not reach the threshold of 20 CD34+cells/mcl in PB, while 29/592 (5%) collected $<2x10^6$ CD34+/kg in $\leq$3 aphereses (16 pts met both the criteria): they were proven PM, according to GITMO criteria (1 of the following: CD34 peak in peripheral blood (PB); final CD34+ collection). The analysis of the 47 proven PM shows a recurrent pattern: low platelet count and advanced/refractory disease; 14% received radiotherapy (extensive in 1 case). Univariate analyses were performed for 17 covariates and 10 (with a significance level $P<0.1$) included in a multivariate logistic regression model; stepwise backward selection was applied, yielding a simplified unadjusted model with 4 covariates (BM infiltration before mobilization, sum of CHT courses, disease type, previous mobilization failure). For the final optimization two composite factors were included, derived from interaction terms: 1-"compromised BM performance", (combination of neoplastic BM infiltration, any degree, and platelet count $<$170,000/mcl before mobilization);2-adjusted sum of CHT courses, where those treatments at high risk for mobilization failure(Melphalan/Lenalidomide/Fludarabine/BCNU/radioimmunoconjugates) were counted twice the others. Based on the coefficients of the final regression model, the following prediction score was generated: **(compromised BM performance x 2.22)+(adjusted sum of CHT courses x 1.20)+(Previous mobilization failure x 3.11)+disease specific score (+0.45 if NHL |+1.65 if MM | -1.2 if HD).** The score varied between 0 and 10 and produced a ROC curve with an AUC 0.7712; setting the cut-off value at 6 yielded a specificity of 94% and a sensitivity of 44% for the outcome of PM.
Conclusion: In our training set, the percentage of mobilization failures appears inferior than in previous historical series (8%); relevance of the GITMO criteria for an a priori identification of the PM has been confirmed by this analysis except for the radiotherapy (maybe due to the very low percentage of pts who actually receive it before mobilization), but a different hierarchy of factors emerged. Excluding the extensive radiotherapy and including the pre-mobilization platelet count, we built a simple score with a very high specificity, even though a relatively low sensitivity. This score allows to identify pts at very high risk of mobilization failure, but it is not sensitive enough to predict failure in pts with low/absent clinical risk factors; in these cases both the dynamic evaluation of the daily CD34+/WBC count and maybe new biological specific markers should be prospectively evaluated.
Disclosure of Interest: None declared.

P412
Abstract Withdrawn

P413
Low VEGF, MMP9 and ANGPT1 levels after autologous hematopoietic stem cell transplantation are associated with shorter time to engraftment

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Introduction: Mateusz Nowicki*, Anna Szmigielska-Kapłon*
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Bone marrow niche, as a site of complicated cytokines interactions, has been the subject of many scientific studies, mainly in the context of the proteins influence on damage or recovery of endothelium after allogeneic hematopoietic stem cell transplantation (HSCT) and during acute GVHD. In our research, we wanted to explore mutual correlations of bone marrow niche cytokines involved in the homing and mobilization of hematopoietic stem cells, as well as in angiogenesis.
The aim of our study was to evaluate the levels of cytokines: osteopontin (OPN), angiopoietin 1 (ANGPT1), angiopoietin 2

(ANGPT2), vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF-1), vascular cell adhesion molecule 1 (VCAM-1) and matrix metalloproteinase 9 (MMP-9) before and after autologous hematopoietic stem cells transplantation and their influence on engraftment.

Materials (or patients) and methods: Twenty four patients were enrolled to the study (16 F, 8 M). The median (Me) age was 56 years. The investigated group consisted of 19 Multiple Myeloma, 4 Non-Hodgkin Lymphoma, and 1 Hodgkin Lymphoma. The blood was collected on 4 time points: before chemotherapy "BC", on the day of HSCT "0" and on the day "+7", "+14" after HSCT. The cytokines were evaluated by ELISA method.

Results: Our study revealed decreased level of ANGPT1, VEGF and MMP-9 on day +7 after HSCT as compared to baseline (BC): ANGPT1 (Me = 1280 vs 2507 pg/ml, $P = 0{,}02$), VEGF (Me = 44 vs 83 pg/ml, $P = 0{,}04$), and MMP-9 (Me = 8 vs 72 ng/ml, $P = 0{,}01$). The cytokines levels increased to baseline values at day +14 after HSCT.

To evaluate influence of cytokines on engraftment time we divided patients according to median cytokines levels into two groups: "high" and "low expressors" (above and below median).

We observed, that in the group of ANGPT1 "low expressors" and MMP-9 "low expressors" on +7 day after HSCT, the time to engraftment was shorter than in "high expressors" (Me = 12 vs 17 days, $P = 0{,}01$ and Me = 12 vs 16 days, $P = 0{,}01$ respectively). At the day 14 after HSCT VEGF "low expressors" as well as MMP-9 "low expressors" had faster engraftment than "high expressors" (Me = 12 vs 16 days, $P = 0{,}046$ for VEGF and Me = 12 vs 16 days, $P = 0{,}03$ for MMP-9).

Our research revealed correlation between both ANGPT1and MMP-9 levels on +7 day after HSCT and the time to engraftment (R = 0,49, $P = 0{,}02$ and R = 0,48, $P = 0{,}02$ respectively). Additionally we found correlation between levels of VEGF as well as MMP-9 assessed on +14 day after HSCT and time of regeneration (R = 0,49, $P = 0{,}04$ for VEGF and R = 0,61, $P = 0{,}008$ for MMP-9).

The OPN, VCAM-1 and SDF-1 levels measured in 4 time points did not differ and no correlation with engraftment was found.

Conclusion: In conclusion, levels of cytokines (VEGF, ANGPT1 and MMP-9) change significantly in the early post-transplant period. Profound decrease of ANGPT1 and MMP-9 at the time of neutropenic nadir correlates with faster engraftment after HSCT.

Disclosure of Interest: None declared.

P414

Blood stem cell graft composition collected after various mobilization methods in myeloma and lymphoma patients

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Introduction: Mobilized peripheral blood stem cells (PBSCs) are the preferred graft for autologous stem cell transplantation (ASCT). The use of cytokines, alone or in combination with chemotherapy, is currently the most common strategy to collect PBSCs. For patients who are hard-to-mobilize, plerixafor (P) has shown to enhance stem cell mobilization more effectively than standard mobilization regimens. However, limited data are available on graft content collected after various mobilization methods. The aim of this study was to assess the effects of different mobilization strategies on CD34 cell subclasses and lymphocyte subsets.

Materials (or patients) and methods: A total of 36 stem cell grafts from patients with multiple myeloma ($n = 18$) and malignant lymphoma ($n = 18$) who were mobilized with G-CSF alone (G; $n = 6$), chemotherapy + G (CG; $n = 13$), G + P (GP; $n = 12$) or chemotherapy + G + P (CGP; $n = 5$) were included in this study. Flow cytometric analyzes were performed in the majority from cryopreserved graft samples by a LSRFortessa™ cell analyzer (Becton Dickinson). For characterization of CD34 subsets, cells where stained according to the protocol of Görgens et al (Cell Reports 2013) and classified as (a) multipotent progenitors (MPP; $CD34^+CD133^+RA^-$),(b) lymphoprimed myeloid progenitors (LMPP; $CD34^+CD133^+RA^+$) which differentiate into multilymphoid progenitors (MLP; from which B-cells and monocytes derive) and progenitors of granulocytes and macrophages (GMP; $CD34^+CD133^-RA^+$), (c) erythromyeloid progenitors (EMP; $CD34^+CD133^-RA^-$) which differentiate into progenitors of megakaryocytes and erythrocytes (MEP) and progenitors of eosinophilic and basophilic lymphocytes (EoBP). In addition, $CD3^+$ and$CD19^+$ lymphocytes were analyzed.

Results: There was no significant difference in the amount of harvested $CD34^+$, and in the proportion of EMP between the groups. The amount of $CD3^+$ lymphocytes was significantly higher in the GP vs chemomobilized groups (CGP + CG, $n = 18$), whereas $CD19^+$ cells showed a trend to be higher after GP mobilization. Interestingly, the proportion of the most primitive stem cells (MPP) was significantly higher in the G group ($n = 6$) compared to patients mobilized with P (GP + CGP, $n = 17$). Chemomobilized patients ($n = 18$) collected a significantly higher proportion of LMPP whereas the proportion of GMP was significantly higher in the CGP group and the proportion of MLP was the highest in the GP group, respectively.

Conclusion: In contrast to previous findings where P reportedly mobilized more primitive stem cells, we found that mobilization by cytokine alone yielded the highest proportion of MPP. Whether these differences are associated with immune reconstitution, long-term engraftment or patient outcome needs to be evaluated in larger patient groups with longer follow-up.

References: Görgens A et al. Revision of the human hematopoietic tree: granulocyte subtypes derive from distinct hematopoietic lineages. Cell Rep. 2013;3(5): 1539-52.

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P415

Blood stem cell graft composition after pre-emptive plerixafor injection in patients who mobilize poorly after mobilization with or without chemotherapy and granulocyte-colony stimulating factor

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Introduction: Routinely, autologous peripheral blood stem cells are mobilized by the use of granulocyte-colony stimulating factor (G-CSF), alone or in combination with chemotherapy. To enhance stem cell release from the bone marrow, plerixafor may be used in patients who mobilize poorly.

Currently, the most common praxis is to add plerixafor after 4 days G-CSF but it can also be used in combination with chemotherapy. Limited data do exist in regard to the compositions of grafts mobilized with plerixafor in addition to G-CSF alone (GP) or chemotherapy (CGP). The aim of this prospective study was to assess the effects of GP and GCP on CD34 cell subclasses and lymphocyte subsets.

Materials (or patients) and methods: A total of 55 stem cell grafts from patients with multiple myeloma ($n = 38$), non-Hodgkin's lymphoma ($n = 15$) or Hodgkin's disease ($n = 2$) who mobilized poorly after G-CSF (71%; GP group) or chemotherapy (29%; CGP group) were included in this study. Flow cytometric analyzes were performed from cryopreserved samples by a LSR Fortessa™ cell analyzer (Becton Dickinson). For characterization of CD34 subsets and lymphocytes, cells where stained according to the protocol of Görgens et al and classified as (a) multipotent progenitors (MPP; $CD34^+$ $CD133^+$ RA^-), (b) lymphoprimed myeloid progenitors (LMPP; $CD34^+$ $CD133^+$ RA^+) which differentiate in multilymphoid progenitors (MLP; from which B-cells and monocytes derive) and progenitors of granulocytes and macrophages (GMP; $CD34^+$ $CD133^-$ RA^+), (c) erythromyeloid progenitors (EMP; $CD34^+$ $CD133^-$ RA^-) which differentiate in progenitors of megacaryocytes and erythrocytes (MEP) and progenitors of eosinophilic and basophilic lymphocytes (EoBP). In addition $CD3^+$, $CD19^+$ lymphocytes and CD4/8 ratio was calculated.

Results: There was no significant difference in the amount of harvested CD34+ cells and in the proportion of the most primitive stem cells (MPP), EMP, amount of $CD3^+$ lymphocytes and CD4/8 ratio between the both groups. The amount of $CD19^+$ lymphocytes was significantly higher in the GP group whereas the proportion of LMPP and GMP was significantly higher in the CGP group.

median (range)	*GP, n = 39*	*CGP, n = 16*	P-*value*
$CD34^+$ x 10^6/kg	2.0 (0.2-11.3)	3.3 (0.2-12.7)	0.421
Proportion of $CD34^+$ $CD133^+$ RA^- %	35(12-71.9)	34.0 (18.1-55.6)	0.653
Proportion of $CD34^+$ $CD133^-$ RA^- %	27.4(13.8-60.2)	22.8(8.0-41.7)	0.077
Proportion of $CD34^+$ $CD133^+$ RA^+ %	19.5(4.8-57.3)	29.0(8.5-42.0)	0.001
Proportion of $CD34^+$ $CD133^-$ RA^+ %	3.3(0-26.5)	9.0(2.9-30.7)	0.008
$CD3^+$ x 10^6/kg	101.9(5.3-901.4)	93.4(4.7-203.3)	0.220
$CD19^+$ x 10^6/kg	6.8(0-231.1)	0.5(0-13.6)	0.012
CD4/8 ratio	0.8(0.2-4.5)	0.6(0.3-2.7)	0.630

Conclusion: In conclusion, we found that plerixafor, when used pre-emptively in addition to cytokine alone or chemomobilization shows no advantage for a respective mobilization regimen with regards to the amount of collected $CD34^+$ cells and proportion of more primitive stem cells. However, the proportion of LMPP and GMP was higher in CGP grafts and the amount of B-lymphocytes in grafts collected after GP, respectively. Whether these differences are associated with immune reconstitution, long-term engraftment, or patient outcomes needs to be evaluated in larger patient groups with longer follow-up.

References: Görgens A et al. Revision of the human hematopoietic tree: granulocyte subtypes derive from distinct hematopoietic lineages. Cell Rep. 2013;3(5): 1539-52.

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P416

Filgrastim- versus chemotherapy-based autologous hematopoietic stem cell mobilization after pre-emptive use of plerixafor for myeloma and lymphoma patients: preliminary data of a non-interventional study

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Introduction: Mobilized peripheral blood stem cells (PBSCs) are the preferred graft for autologous stem cell transplantation (ASCT). The use of cytokines, alone or in combination with chemotherapy, is currently the most common strategy to collect PBSCs. However, a significant proportion of patients is hard-to-mobilize. Plerixafor (P) has recently been introduced to enhance PBSC mobilization and has been shown to be more effective than filgrastim (G-CSF) alone in patients with multiple myeloma (MM) or non-Hodgkin's lymphoma (NHL). Data on combining plerixafor with chemotherapy plus G-CSF in patients who mobilize poorly are limited. The aim of this non-interventional, prospective, study was to observer the efficacy of a pre-emptive use of plerixafor after G-CSF (GP) or chemomobilization (GCP) in terms to achieve $>2x10^6$/kg CD34+ cells, thus, enabling patients to be eligible for ASCT. Additionally grafts were analyzed with respect to CD34+ cell subclasses and lymphocyte subsets.

Materials (or patients) and methods: Between June 2012 and October 2014, 84 patients with MM ($n = 54$), NHL ($n = 27$) or Hodgkin's disease (HD, $n = 3$) who mobilized poorly after G-CSF (53%; GP group) or chemomobilization (46%; CGP group) were included in a prospective, non-interventional study. Plerixafor was given in case of CD34+ <20cells/μl in the peripheral blood in the GP group on day 4 of G-CSF and in the CGP group on the anticipated collection day if leucocytes reached ≥5.000/μl. The majority of patients underwent large volume apheresis processing 3.5-4 times total blood volume.

Results: A total of 98 mobilization regimens were applied. With the preemptive use of P it was possible to collect sufficient PBSC in 80 of 84 (95%) patients and in 82 of 98 (80%) mobilization attempts. The mean amount of CD34+ cells in the peripheral blood before P was was 6.6/μL (SD ± 5; range 0-19.7) did not differ between GP (6.0/μL ± 5; range 0-19.7) or CGP (7.4/μL ± 5; 0.7-17.0), respectively. After P, blood CD34+ cells showed a marked increase to 31.7/μL (SD ± 28; range 0-175) but did not different between GP (30.9 ± 25 range 2.3-83.7) or CGP (30.2 ± 31; 1.6-175.0) resulting in a mean amount of 4.7 (± 3.7; range 0.23-31.34) x10^6/kg CD34+ cells. The number of harvested CD34+ cells did not differ between the GP (4.5 ± 2.6 x10^6/kg) and GCP (4.8 ± 4.8 x10^6/kg) group, respectively.

Conclusion: In conclusion, circulating CD34+ cell counts can be significantly increased with P after G-CSF or chemomobilization, showing no advantage for a particular regimen (GP vs. CGP) and the majority of patients considered difficult to mobilize can be successfully collected. Possible differences in graft composition depending of the mobilization regimen used (GP vs. CGP), and an influence on time to engraftment, immune reconstitution and risk of relapse after ASCT need to be evaluated in further studies.

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P417

Negative depletion approaches of CD3 αβ t lymphocytes in haploidentical transplant in thalassemia and sickle cell disease patients

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Introduction: 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. 43 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 25 patients, and 200 mg/kg cyclophosphamide,10 mg/kg Thiotepa, and 10 mg/kg ATG (Fresenius) daily from day -5 to -2. 34 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 2x105/kg by fresh marrow cell add back at the time of transplant. Both groups received cyclosporine for graft versus host disease prophylaxis for two months post transplant. 4 patients died, 12 patients reject their grafts, and 27 showed full chimerism with functioning grafts at a median follow-up of 56 months. The overall survival, thalassemia free survival and mortality is 90%; 59% and 11%.To analyze immunohematologic reconstitution, particularly NK cells, we evaluated 13 thalassemia patients after 20 and 60 days and 1 year post transplantation with T cell-depleted HLA-haploidentical stem cells. NKs were the first lymphocytes to repopulate the peripheral blood. A significant increase in CD4+ and CD8+ markers paralleled an increase in CD3-CD16+ NKs, especially with full engraftment,suggesting a role for newly generated NK cells in improved engraftment and in prevention of rejection by an attack of the host lympho-hematopoietic cells
Materials (or patients) and methods: In order to increase the T-cell depletion of PBSC while maintaining the anti-infectious and the engraftment facilitating effects of the depleted grafts, we have introduced the depletion of αβ T Lymphocytes using the CliniMacs System.In addition to the Cd3 depletion, αβ-depleted grafts contain large number of γδ T lymphocytes.-studies were then conducted to determine if γδ T cells were capable of preventing graft rejection in the context of haploidentical transplant.Using this new strategies, with the same conditioning regimen, in 10 haploidentical transplant, age 3-12, 7 thalassemia, 3 sickle cell.
Results: **Results**:9 patients are disease free, 8 patients engrafted persistently in 1/9 cases we observed, after transplant failure, reactivation of HbF,the patient remains free of trasfusion therapy,1 patient rejected the transplant.**GVHD**: 3 patients showed CGVHD.**In term of immunological reconstitution** after 60 days post transplant, we observed an increase of T cells (CD4+ and CD8+), B cells (CD19+) and NK cells (especially CD3-CD16+, with cytotoxic potential) in the peripheral blood of these patients.

[P418]

Table 1. Values are expressed in median (range)

	Day of myeloid engrafment	*Day of engrafment platelet*	*Fever Days*	*Days in Hospital*	*RBC transfusion*	*Platelet transfusion*
<10CD34+/μL	11 (9-14)	12 (6-37)	3 (0-37)	23 (16-37)	2 (0-8)	33 (0-110)
≥10CD34+/μL	11 (8-25)	12 (6-36)	3 (0-48)	23(16-108)	2 (0-30)	30 (0-140)
p	0.716	0.131	0.448	0.154	0.815	0.740

Conclusion: In conclusion, this abstract has illustred how full haplotype-mismatched transplantation has evolved to an established form of treatment that could be considered for patients affected by nonmalignant disease like thalassemia major and sca.
Disclosure of Interest: None declared.

P418

Autologous peripheral blood stem cell transplantation in poor mobilizer patients

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Introduction: Pre-apheresis peripheral blood $CD34^+$ cell count ($PBCD34^+$) predicts the yield of the leukapheresis. Patients with pre-apheresis PBCD34+ lower than 10 $CD34^+$/μL are considered poor mobilizer patients (PMP) and they do not undergo leukapheresis in most institutions; therefore about 20% of patients requiring autologous stem cell transplantation (ASCT) are not transplantated.
Our objective was to study the engraftment of ASCT obtained by large-volume-leukapheresis (LVLKA) in PMP compared with "good mobilizers" (GM).
Materials (or patients) and methods: A total of 169 ASCT were included: 52 Multiple Myeloma (MM), 41 breast cancer (BC), 41 non Hodgkin lymphoma (NHL), 11 Hodgkin disease (HD), 8 acute myeloid leukemia (AML), 7 chronic lymphocytic leukemia, 5 acute lymphoblastic leukemia, 1 chronic myeloid leukemia, 3 other. Pre-apheresis $PBCD34^+ < 10/\mu L$ was found in 60 patients (35.5%), 16 MM, 18NHL, 7 BC, 6HD, 4AML and 9 other diagnoses.
To compare PMP with GM we analyzed the following parameters: number of apheresis required to obtain $\geq 2x10^6 CD34^+$ cells / kg receptor weight, final infused volume in ASCT, final CD34+ infused dose, final infused GM-CFU and graft kinetics. Myeloid and platelet engrafment were defined as the first day of neutrophil count $> 0{,}5x10^9$/L and platelet count $> 20x10^9$/L respectively postransplant. We also compared hospitalization days (HD), days of fever and transfusion requirements of red cells concentrates (RBC) and units of platelets.
Results: In the group of PMP a median 3 LVLKA (1-9) were required to obtain >= 2x10^{6}CD34+ cells/Kg vs. 2(1-7) LVLKA required in the GM group ($P < 0.001$).
The final cell dose in PMP was 549 mL (136-2922 mL) vs. 288 mL (6.2-2000 mL) in the GM group ($P < 0.001$). Infused CD34+ dose was 2.42x10^6/Kg (1.51-7.7x10^6/Kg) in the group of PMP vs. 4.17x10^6/Kg (1.51-32 x106/Kg) in the others ($P < 0.001$). However infused GM-CFU content did not reach statistical difference between the PMP 0.93 x10^4/Kg (0.93-37.06 x10^4/Kg) vs. GM 2.8 x106/Kg (0.0-41.7 x10^4/Kg). The engraftment results are shown in the table.
Conclusion: In poor mobilizer patients enough amount of CD34+ cells for ASCT can be collected with LVLKA. Our data suggest that pre-apheresis PBCD34+ count should not be an exclusion criteria for patients needing an ASCT.
Disclosure of Interest: None declared.

P419

Autologous peripheral blood stem cell transplantation in very poor mobilizer patients

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Introduction: Pre-apheresis peripheral blood CD34+ cell count (PBCD34+) predicts the yield of the leukapheresis. Patients with pre-apheresis PBCD34+ lower than 10 CD34+/μL are considered poor mobilizers patients (PMP), some patients present less than 5 CD34+/μL pre-apheresis count, considered very poor mobilizer patients (VPMP). The usual trend in these patients is to suspend the leukapheresis and this is one of the reasons why about 20% of patients requiring autologous stem cell transplantation (ASTC) are not finally transplanted.

Our objective was to compare the apheresis product and engrafment parameters obtained large-volume-leukapheresis (LVLKA) in VPMP compared PMP.

Materials (or patients) and methods: A total of 169 ASCT were included: 52 Multiple Myeloma (MM), 41 breast cancer (BC), 41 non Hodgkin lymphoma (NHL), 11 Hodgkin disease (HD), 8 acute myeloid leukemia (AML), 7 chronic lymphocytic leukemia, 5 acute lymphoblastic leukemia, 1 chronic myeloid leukemia, 3 other. Pre-apheresis PBCD34+ < 5/μL was found in 28 patients (16.5%), 9MM, 9NHL, 2BC, 2AML and 4 other diagnoses.

To compare PMP with GM we analyzed the following parameters: number of apheresis required to obtain ≥ $2x10^6$CD34+ cells / kg receptor weight, final infused volume in ASCT, final CD34+ infused dose, final infused GM-CFU and graft kinetics.

Mieloyd and platelet engrafment were defined as teh first day of neutrophil count > $0,5x10^9$/L and platelet count > $20x10^9$/L respectively postransplant. We also compared hospitalization days (HD), days of fever and transfusion requirements of red cells concentrates (RBC) and units of platelets.

Results: In the group of VPMP a median of 3 (2-9) LVLKA were required to obtain $2x10^6$CD34+ cells/Kg vs. 2(1-7) LVLKA required in GM group ($P < 0,001$).

The final volume infused in VPMP was 692 ml (136-2109 mL) vs. 326 ml (6.2- 2922 mL) in GM group ($P < 0.001$). Infused CD34+ cell dose was $2.2x10^6$/Kg (1.8-5.8 $x10^6$/Kg) in the group of VPMP vs. $3.16x10^6$/Kg (1.51-32 $x10^6$/Kg) in GM ($P < P < 0.001$). However infused GM-CFUcontent did not reach statistical difference between the PMP 0.93 $x10^4$/Kg (0.93-37.06 $x10^4$/Kg) vs. GM 2.8 $x10^4$/Kg (0.0-41.7 $x10^4$/Kg) in the other group. The engraftment results are shown in the table.

Table 1. Values are expressed in median (range)

	Day of myeloid engrafment	*Day of platelet engrafment*	*Fever Days*	*Days in Hospital*	*RBC transfusion*	*Platelet transfusion*
<5 CD34+/μL	12 (9-14)	12 (8-31)	3 (0-12)	23 (18-37)	2 (0-8)	33 (0-110)
≥5 CD34+/μL	11 (8-25)	12 (6-37)	3 (0-48)	23 (16-108)	2 (0-30)	30 (0-140)
p	0.253	0.246	0.036	0.123	0.539	0.581

Conclusion: In very poor mobilizer patients enough amount of CD34+ cells for ASCT can be collected with LVLKA. Our data suggest that pre-apheresis PBCD34+ count should not be an exclusion criteria for patients requiring an ASCT.

Disclosure of Interest: None declared.

P420

Use of upfront plerixafor to enable successful haematopoietic stem cell mobilisation in dialysis dependent patients with multiple myeloma: a single tertiary centre's experience

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Introduction: Use of plerixafor for haematopoietic stem cell (HSC) mobilisation has been described in patients with varying degrees of renal failure including dialysis dependent patients. We have adopted the practice of using Granulocyte Colony Stimulating Factor (GCSF) with or without plerixafor upfront to mobilise dialysis dependent patients with multiple myeloma (MM) as a convenient method of scheduling harvest around dialysis.

Materials (or patients) and methods: We retrospectively screened our hospital's transplant database for patients who underwent autologous haematopoietic stem cell transplant (auto-HSCT) for MM from 2009 onwards. 7 patients who were planned to have upfront GCSF/plerixafor mobilisation for HSC harvesting were identified and included. The schedule involved the use of GCSF at a dose of 10 mcg/kg from day -7 to day -4. CD34 positive cell count was checked on day – 4 to determine whether plerixafor was needed and the patients were dialysed. Stem cells were collected on day -3 and -2. Melphalan was given on day -2 and patients received dialysis on days -1 and 0 following which, fresh stem cells were re-infused. Further dialysis was given as clinically indicated. Data were gathered on patient demographics, count of CD34+ cells obtained, progression or not to transplant, time to platelet and neutrophil engraftment, infective complications and length of stay. Overall time to engraftment and length of stay for all patients undergoing auto-HSCT for MM was also looked at.

Results: The average age of the patient group receiving GCSF/plerixafor upfront was 60.7 years. 6 patients needed GCSF and plerixafor and 1 patient achieved adequate CD34+ counts with only GSCF. All but one patient had fresh cells re-infused. All patients had a successful stem cell harvest. Mean count of CD34+ cells collected was 4.56 x 10^6/kg. Median time to neutrophil engraftment defined as neutrophil count more than 0.5 x 10^9/L was 11 days and median time to platelet engraftment defined as platelet count more than 50 $x10^9$/L was 18 days. Median length of stay measured from the day of transplant was 19 days. All patients but one required intravenous antibiotics although none required admission to the intensive care unit. In comparison, the median time to engraftment for all patients undergoing auto-HSCT for MM in our centre from 2009 to present was 15 days for neutrophil engraftment and 27 days for platelet engraftment (171 patients in total excluding patients discussed above). Median length of stay for these patients was 19 days after day of transplant.

Conclusion: Our data support the use of GCSF with upfront plerixafor to enable successful HSC mobilisation with subsequent re-infusion of fresh cells in patients with MM on dialysis. In our experience satisfactory CD34+ cell counts have been obtained and cancellations or delays avoided. Although concerns have previously been raised that patients may be slow to engraft resulting in prolonged inpatient stay and increased cost we have found this not to be the case in our practice. The high rate of infective complications is probably a reflection of co-morbidities observed in this patient group rather than the mobilisation method. We suggest that the above schedule is a convenient and effective method of HSC mobilisation in this patient group although further data should be collected.

Disclosure of Interest: None declared.

P421

Mobilization of autologous and allogeneic peripheral blood stem cells for stem cell transplantation using biosimilar G-CSF – An Up-date

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Introduction: Biosimilars of the granulocyte colony stimulating factor (G-CSF) filgrastim were approved by the European Medicines Agency (EMA) for registered indications of the originator G-CSF (Neupogen®) including prevention and treatment of neutropenia as well as mobilization of peripheral blood stem cells. Nevertheless there is still an ongoing debate regarding quality, efficacy and safety of biosimilar G-CSF.
Materials (or patients) and methods: This abstract is an update of our comprehensive review on the use of biosimilar G-CSF. Patients with different hematological malignancies were covered for the autologous setting. Healthy donors that were mobilized at multiple centers using biosimilar G-CSF in the allogeneic setting.
Results: More than 1200 patients mostly with hematological malignancies as well as healthy donors were successfully mobilized for autologous or allogeneic stem cell transplantation using biosimilar G-CSF (Ratiograstim®/Tevagrastim: 709 patients; Zarzio®:, 520 patients). 464 patients with multiple myeloma, 331 with Non-Hodgkin's lymphoma (NHL), 104 with Hodgkin's lymphoma (HL), and other disease were included in this review. Biosimilar G-CSF was also used to mobilize hematopoietic stem cells in 230 sibling or volunteer unrelated donors.In both groups using either the originator G-CSF or biosimilar G-CSF similar results were seen for the following parameters: (1) yield of CD34+ stem cells, (2) clinical side effects in the donor, (3) engraftment of the transplant, (4) side effects in the patient.
Conclusion: In summary, the biosimilar and originator G-CSFs are highly similar in terms of PBSC yield as well as their toxicity profile are equivalent to historical data.
Disclosure of Interest: None declared.

P422

Increased efficacy of intermediate-dose cytarabine + G-CSF compared to DHAP + G-CSF for stem cell mobilization in patients with lymphoma: an analysis from the Polish Lymphoma Research Group

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Introduction: Autologous HCT is a standard of care for lymphoma patients who fail initial conventional-dose chemotherapy. Salvage regimens supported with G-CSF are frequently used for stem cell mobilization. Among treatment schedules, DHAP (dexamethasone, cytarbine, cisplatin) +/- rituximab is one of the most popular, however, it is associated with significant risk of mobilization failure. In recent years intermediate-dose cytarabine (ID-AraC) + G-CSF has been proposed an alternative schedule (Giebel et al, Bone Marrow Transplant 2013, 48: 915-21). The aim of this multicenter, retrospective study, performed by the Polish Lymphoma Research Group, was to compare the efficacy and safety of both regimens.
Materials (or patients) and methods: 101 consecutive patients with Hodgkin's lymphoma ($n = 56$) or non-Hodgkin lymphoma ($n = 45$) who had received at least 2 lines of chemotherapy, mobilized between 2011-2013 with either DHAP(+/-R) ($n = 51$) or ID-AraC ($n = 50$) + G-CSF were included in the analysis. AraC was administered at the dose of 400 mg/m2 bid for 2 days (total dose 1600 mg/m2) followed by filgrastim 5-10 ug/kg starting from day 5. Both groups did not differ with respect to age, the diagnosis, disease stage, number of preceding lines and courses of chemotherapy as well as administration of radiotherapy.
Results: In the AraC group, 48 patients (96%) collected at least 2 x 10^6 CD34+ cells/kg compared to 36 patients (71%) in the DHAP group ($P = 0.0006$). The median peak number of circulating CD34+ cells was 61 (0-538)/μL vs. 37 (0-396)/μL ($P = 0.02$), while the median number of collected CD34+ cells was 9.3 (0-30.3) x10^6/kg vs. 5.6 (0-24.8) x10^6/kg, respectively ($P = 0.006$). A single apheresis was sufficient to achieve the threshold number of CD34+ cells in 41 cases (82%) after AraC compared to 23 (45%) after DHAP ($P = 0.001$). The median day of the first apheresis was 14 (range, 12-18; SD = 1.2) after ID-AraC and 15 (11-23, SD = 2.5) after DHAP.
In a multivariate analysis adjusted for age and the number of preceding lines of chemotherapy, the use of AraC as compared to DHAP was associated with significantly increased chance of collecting at least 2 x 10^6 CD34+ cells/kg (OR = 13.1, 95%CI, 2.6-66; $P = 0.002$).
None of the patients treated with ID-AraC developed febrile neutropenia compared to 2 (4%) patients in the DHAP group ($P = 0.16$). There were no cases of treatment-related mortality. In the ID-AraC group, 29 (58%) patients required transfusion of platelets and 13 (26%) needed transfusion of red blood cells. In the DHAP group, the respective proportions were 43 (84%) ($P = 0.003$) and 20 (39%) ($P = 0.16$).
Conclusion: As compared to DHAP + G-CSF, the use of ID-AraC + G-CSF for stem cell mobilization is associated with significantly higher efficacy, allowing for collection of the transplant material in almost all patients with lymphoma, usually with a single apheresis. It appears safe, less frequently requires platelet transfusions and is highly predictable in terms of the timing of stem cell harvest. This observation suggests that for lymphoma patients treated DHAP as a salvage regimen last course may be substituted by ID-AraC monotherapy in order to increase a chance of stem cell collection.
Disclosure of Interest: None declared.

P423

Age, Cytopenia at Diagnosis and History of Hypertension are associated with Stem Cell Mobilization Failures Among Patients with Multiple Myeloma

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Introduction: The GIMEMA group has recently developed a risk score predicting mobilization failure for patients with Multiple Myeloma (MM) (Musto et al, EHA 2014). The risk score included age, cytopenia at diagnosis, induction therapy agents and their hematological toxicity. In this study we aimed to evaluate the impact of these factors on peripheral blood stem cell (PBSC) mobilization among our patients who receive different induction regimens.
Materials (or patients) and methods: Total 178 newly diagnosed patients with MM (M:110, F:68) planned for autologous SC transplantation from two different centers; median age 56 years (30-70) were included in the analysis. This retrospective study examined the impact of age (>60 years), gender, cytopenia at diagnosis, hematological toxicity during induction therapy, the type of induction and mobilization methods, the presence of neuropathy or comorbid diseases

(diabetes mellitus, hypertension and renal failure), the use of beta blocker drugs on mobilization success. Patients with $CD34^+$ levels of $<20/\mu L$ in peripheral blood at maximum stimulation were considered to be poor mobilizers. The total amount of PBSC $<5x10e6/kg$ after a single mobilization procedure was defined as sub-optimal collection.

Results: Optimal mobilization with median two apheresis (1-4) sessions for PBSC was obtained in 82.9% of the patients ($n = 145$). Median $CD34^+$ cells in this group were 8.33x10e6/kg (5-27x10e6/kg). Poor mobilization was observed in only two patients ($<2x10e6/kg$). Optimal CD34+ cells on the first collection day was not achievable among those with advanced age compared to younger patients (43.1% vs 26.4%, $P = 0.038$). In addition, the presence of cytopenia at diagnosis was the only significantly detrimental factor on optimal collection (87.4% vs 74.1%, $P = 0.03$). Poor mobilisation in the first collection was observed in the patients with history of hypertension (26.5% vs 74.5%, $P = 0.012$). Hypertension was an independent predictor not associated with age. In multivariate analysis, age and the use of G-CSF alone for the SC mobilisation continued to be detrimental at the first day collection ($P = 0.38$ and <0.0001). Besides, cytopenia at the diagnosis had a negative impact on the total collection in multivariate analysis ($P = 0.037$).Table: Summary of results listing the factors analyzed.

	Optimal collection (first day)	P	*Total collection*	P
Age >60 vs. <60	26.4% vs 43.1%	0.038*	83.6% vs 83.2%	0.94
Neutropenia at diagnosis + vs -	29.8% vs 40.4%	0.19	74.1% vs 87.4%	0.033*
Bortezomib at induction + vs -	36.8% vs 38.2%	0.84	80.3% vs 84.6%	0.46
Neuropathy + vs -	37.8% vs 37.9%	0.99	84.5% vs 82.5%	0.58
Cytopenia during induction	31.1% vs 39.6%	0.28	83.9% vs 82.0%	0.76
Use of beta blockers	50.0% vs 36.9%	0.36	83.3% vs 83.3%	1.0
Presence of diabetes mellitus	23.1% vs 39.1%	0.25	84.6% vs 83.2%	0.89
History of hypertension	35.3% vs 38.5%	0.73	79.4% vs 84.3%	0.49
G-CSF alone or with chemotherapy	27.2% vs 53.7%	<.0001*	82.2% vs 83.9%	0.78

Conclusion: In our experience, 17.1% of the myeloma patients showed suboptimal or poor mobilization. Use of bortezomib, age, presence of neuropathy or hematological toxicity during induction did not significantly impair mobilization. However history of hypertension and also, in correlation with Musto's findings, advanced age or cytopenia at diagnosis were associated with a trend to failure.

Disclosure of Interest: None declared.

P424

Plerixafor-based salvage of patients with failed CD34 + stem cell mobilization achieves comparable results to first-line protocols

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Introduction: Mobilization of CD34+ cells into peripheral blood for autologous hematopoietic stem cell transplantation (SCT) is traditionally accomplished by growth factor alone (G-CSF) or by chemo-mobilization (G-CSF plus chemotherapy). However, a significant proportion of patients are hard to mobilize with these schemes. Plerixafor is a CXCR4 chemokine receptor antagonist for use in combination with G-CSG in patients with lymphoid malignancies who are poor or predicted poor mobilizers.

We aim to determine whether plerixafor can be an effective solution for poor mobilizers, by comparing patients who underwent autologous stem-cell mobilization using plerixafor as the first-line mobilization protocol, with patients who were treated with plerixafor after one or more prior unsuccessful mobilizations with non-CXCR4-based protocols.

Materials (or patients) and methods: We analysed the records of 55 patients who underwent CD34+ mobilization with plerixafor (0.24 mg/kg) plus G-CSF (10 μg/kg) between 10/01/2008 and 12/09/2014.

Results: We evaluated 55 patients (30M), with a median age of 52 years old (16-66). There were 11 Hodgkin's Lymphomas (HL), 15 Non-Hodgkin's Lymphomas (NHL) and 15 Multiple Myelomas (MM). Patients were treated with a median of 2 prior regimens (1-6) and 18 underwent radiotherapy. At least one attempt at CD34+ mobilization with chemotherapy + G-CSF (61.1%) or G-CSF alone (38.9%) was performed in 36 patients. Plerixafor was pre-emptively administered in 19 patients.

We found that the delta increase in the absolute number of CD34+ cells in peripheral blood (by flow cytometry) was not different between the pre-emptive (9.2 ± 1.5) and secondary (13.8 ± 3.1 cells/mL, $P = NS$) approaches; the ratio of increase (3.6 ± 0.3 vs 4.9 ± 0.9 fold, $P = NS$) was also similar for the two approaches. The number of aphereses necessary to collect an adequate number of cells for transplantation was identical (1.71 ± 0.14 vs 1.71 ± 0.16, $P = NS$).

Considering engraftment success, there were no differences in the time to engraftment (TTE) of neutrophils after autologous CD34+ stem cell transplantation between patients with pre-emptive and secondary plerixafor (12.4 ± 0.3 vs 13.2 ± 0.2 days, $P = NS$), nor were there differences in the platelet TTE (17.4 ± 1.2 vs 17.7 ± 1.1 days, $P = NS$).

These results were confirmed by multivariate analysis; previous radiotherapy, the number of previous therapeutic lines, the age at plerixafor administration and stem cell collection, and the diagnosis did not influence the three main outcomes (number of aphereses, neutrophil TTE and platelet TTE).

Conclusion: We found that CXCR4 antagonism with plerixafor was highly effective at salvaging patients who had previously failed mobilization, achieving outcomes that were identical to pre-emptively treated patients, both in terms of the increase in CD34+ cells in peripheral blood (a mean of over 10 cells/mL, corresponding to a nearly 5-fold increase over baseline) and the number of aphereses needed to achieve a successful collection. The time to successful engraftment of the collected cells, as determined by peripheral blood neutrophil and platelet counts, was also identical in first- and second-line plerixafor.

Our results thus show that plerixafor can overcome failures in stem-cell mobilization, giving patients with previous unsuccessful mobilizations a chance at a successful autologous stem cell transplantation that is on par with untreated patients.

Disclosure of Interest: None declared.

P425

Increased efficacy of chemomobilization with intermediate dose cytarabine + G-CSF compared to G-CSF alone for patients with multiple myeloma. Interim analysis of a prospective, randomized trial

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Introduction: Double autologous hematopoietic stem cell transplantation (autoHSCT) is widely used for the treatment of multiple myeloma (MM). The procedure requires collection of at least 5 x 10^6 CD34+ cells/kg. Randomized trials comparing chemomobilization with the use of cyclophosphamide (CY) + G-CSF to G-CSF alone did not demonstrate clear advantage of the addition of CY to growth factor. In recent years, intermediate-dose cytosine arabinoside (ID-AraC) + G-CSF has been proposed, showing high mobilization potential, as demonstrated in retrospective analysis (Giebel et al, Bone Marrow Transplant 2013, 48: 915-21). The goal of this prospective, randomized trial (ClinicalTrials.gov identifier: NCT01908621) was to compare the efficacy of ID-AraC + G-CSF with G-CSF alone in patients with MM referred for double

autoHCST. We present results of a planned interim analysis after recruitment of 60 out of 90 patients.
Materials (or patients) and methods: Inclusion criteria were as follows: 1) the diagnosis of MM, 2) age 18-65 years, 3) at least partial remission after one or more lines of therapy including six or more cycles containing IMiDs, bortezomib or melphalan, 4) planned double autoHSCT.
The primary study end-point was the proportion of patients with stem cell yield of at least 5 × 10^6/kg.
Mobilization regimens were as follows: 1) G-CSF arm - filgrastim 10 μg/kg/day for five consecutive days, 2) ID-AraC arm - AraC 2 x 0.4 g/m2 on days 1,2 (total 1.6 g/m2) + filgrastim 10 ug/kg/day starting from day 5. Leukaphereses were started when the level of circulating CD34 + cells reached at least 10/uL and were continued for the maximum three days or until collecting the target CD34 + cell yield. Leukaphereses were performed using Spectra-Optia Apheresis System (TherumoBCT Inc, Lakewood, CO, USA), processing 2 total blood volumes.
Between III.2013 and X.2014, 30 patients were randomly assigned to each study arm. The groups did not differ significantly in terms of patient age [median 60 years, range (48-65) for G-CSF and 56 years (41-65) for ID-AraC] and the number of preceding lines of chemotherapy.
Results: The level required to start leukaphereses was achieved in all pts in the ID-AraC arm and in 28/30 pts in the G-CSF arm. In the ID-AraC group, all 30 patients collected at least 5 x 10^6 CD34 + cells/kg compared to 21 patients (70%) in the G-CSF group ($P = 0.001$). The median peak number of circulating CD34 + cells was 280 (11-1044)/μL vs. 46 (1-150)/μL ($P < 0.00001$), while the median number of collected CD34 + cells was 15.6 (7-38.6) x10^6/kg vs. 5.9 (0-10.6) x10^6/kg, respectively ($P < 0.00001$). A single apheresis was sufficient to achieve the threshold number of CD34 + cells in 24 cases (80%) after AraC + G-CSF compared to 10 (33%) after G-CSF alone ($P = 0.0003$). The median day of the first apheresis was 13 (12-15; SD = 0.65) after ID-AraC.
Conclusion: Mobilization with ID-AraC + G-CSF is associated with significantly higher efficacy than G-CSF alone. In the studied group it allowed for collection of CD34 + cell number adequate for double autoHSCT in all MM cases, usually with a single leukapheresis. This study provides the first evidence coming from prospective, randomized trial for the advantage of chemomobilization over G-CSF monotherapy in terms of the proportion of patients achieving CD34 + yield sufficient for autoHSCT.
Disclosure of Interest: None declared.

P426
Hematopoietic stem and progenitor cell composition in peripheral blood and in mobilized CD34 + cells harvests
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Introduction: Hematopoietic stem cell transplantation is a procedure now well established, although optimal outcomes are not always achieved. Nowadays, mobilized peripheral CD34 + cells are the preferred source of hematopoietic stem and progenitor cells for transplantation purposes. Unfortunately, very little is known about the peripheral CD34 + cells composition in terms of committed myeloid progenitors and stem cells. In the present study, we intend to investigate the proportions of hematopoietic progenitors and stem cells available in mobilized peripheral blood (PB) and correlate this with clinical information and outcomes of patients who underwent a transplantation procedure.
Materials (or patients) and methods: Multicolor flow-cytometry was used to analyze CD34 + cells from 4 bone marrow (BM) samples and 9 PB samples from healthy volunteers and 32 mobilized PB samples from hematological patients prior CD34 + cell harvesting.
Results: RESULTS: Common myeloid progenitors (CMP) were present in a higher percentage in PB compared to BM (47.8% ± 9.5 versus 27.6% ± 9.5 of CD34 + cells) while granulocyte-macrophage progenitors (GMP) were lower in PB compared to BM (10.3% ± 6.9 versus 23.8% ± 7.2). No significant differences were noticed between PB and BM hematopoietic stem cells (HSC). According to literature, progenitor fractions were equally distributed in BM (27.6% ± 9.5 CMP, 23.8% ± 7.2 GMP and 27.6% ± 16.2 megakaryocyte-erythroid progenitors, MEP). No differences in subpopulations fractions were shown between baseline and mobilized CD34 + cells. Concerning the two samples mobilized with the CXCR4 inhibitor Plerixafor instead of G-CSF only, we noticed that a more elevated ratio of GMP were released in PB: 37.8% in patient#1 and 33.8% in patient#2 compared to the average 16.31% of "G-CSF only" mobilized samples. Analyzing CXCR4 levels among subpopulations of both mobilized or unmobilized samples, it was more expressed on GMP than on the other CD34 + cell subsets.
A strong correlation was observed between the number of peripheral CD34 + cells and the number of circulating CMP whose proportion did not change with increasing CD34 + cell release. White blood cells (WBC) count exhibited a significant correlation with the number of mobilized HSC; on the contrary, WBC, hemoglobin and platelet levels did not show correlations with the number of mobilized CMP/GMP/MEP.
We then looked at possible relationships between the number of re-infused subpopulations and the hematological recovery after an auto-transplantation conditioned by high dose chemotherapy. A tendency to inverse correlation was shown between the number of re-infused progenitors and the days of aplasia, as well as between the number of re-infused MEP and erythrocyte/platelet transfusions. However, these results did not reach a statistical significance, probably for the too low patient number.
Conclusion: CD34 + cell subset composition shows differences between BM and PB. We do not know yet if variabilities in the proportions of different progenitor/stem cell re-infused can influence clinical issues such as infections complications and transfusion requirement in patient undergoing an hematopoietic stem cell transplantation. A deep understanding of these mechanisms may guide the clinician in the choice of the most suitable chemotherapy or mobilizing regimen and lead up to an improved clinical outcomes of such patients.
Disclosure of Interest: None declared.

Apheresis stem cell collection and processing

P427
Stem cell apheresis using the Spectra Optia CMNC protocol – Experience of Fundeni Clinical Institute, Bucharest, Romania
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Introduction: In Fundeni Clinical Institute 10 stem cell apheresis were done using the recently released CMNC protocol on Spectra Optia. We describe results of 9 autologous and 1 allogeneic donor collected between 7th October 2014 and 25th November 2014.
Materials (or patients) and methods: 6 myeloma patients were mobilized in the same way (Cy + G-CSF); from this group one patient needed 2 procedures; 1 patient with Hodgkin's Disease was mobilized with IGEV + G-CSF; 1 patient with Ewing Sarcoma was mobilized with Cy + Eto + G-CSF; 1 allogeneic donor was mobilized with G-CSF. For all procedures

a femoral access line was used. Larger blood volumes were processed 9256 ml- 18052 ml.
Results: 1. Relatively light collect line haematocrits were targeted resulting in low total red cell contamination in the final products (median 3.7 mL; range 1.4–6.5).
2. Despite the larger volumes of blood processed, platelet loss to the product was low with a median platelet collection efficiency (CE1%) of 12.8% (Range 8.3% - 20.9%)
3. CD34+ Collection efficiency (CE2%) was 47.4% (Range 34.4%–56.5%)
4. Good total numbers of CD34+ cells were collected from each procedure with each apheresis collection yielding >2.5 x 10^6/kg. Overall data showed a median CD34+ dose of 7 x 10^6/kg (Range 2.80–8.11 x 10^6/kg) per procedure
5. Plotting pre-apheresis CD34/uL versus CD34+ yield per litre of blood shows a good correlation between these variables (R^2 0.76) and accounts for the variable volumes of blood processed in each procedure. This allows an investigator to predict how much blood they may need to process to acquire a target CD34+ dose. Note that one outlier from the linear regression plot was for the single allogeneic donor, who also had an unusually low collection efficiency of 24%. In this case, with a high pre-apheresis WBC of 52.7, a collect pump flow rate of 0.8 mL/minute was used. It may well be that increasing collect rate to 1.0 or greater would improve the extraction efficiency here and correct this.
6. In several cases both the post apheresis PLT and WBC count were actually higher than in the pre-count. It does appear that there is some leucocyte and possibly platelet mobilization during apheresis which might account for these increases.
Conclusion: In conclusion, for CMNC procedures the platelets loss to the product is low which is an advantage for patients and we can predict what is the volume of blood which needs to be process to acquire a target CD34+ dose.I am very sorry for the mistake that I have done and I hope that you can help me.
Disclosure of Interest: None declared.

P428
G-CSFplus preemptive plerixafor versus cyclophosphamide plus G-CSF for autologous stem cell mobilization in multiple myeloma: effectiveness, safety and cost analysis

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Introduction: The optimal stem cell mobilization regimen for patients (pts) with multiple myeloma (MM) remains undefined. Very few studies compared efficacy, toxicity and cost-effectiveness of stem cell mobilization with cyclophosphamide (CY) and granulocyte-colony stimulating factor (G-CSF) versus G-CSF with preemptive plerixafor. We retrospectively compared our single center experience in 89 MM pts using fractionated high-dose CY and G-CSF as our past preferred chemo-mobilization strategy in MM pts with our new mobilization strategy using G-CSF plus preemptive plerixafor.
Materials (or patients) and methods: Pts in the CY group ($n=62$) received either fractionated high-dose CY ($n=56$) (5 g/m^2 divided in 5 doses of 1 g/m^2 every 3 hours) or CY at 50 mg/kg/day for 2 doses ($n=6$). G-CSF was started on day +6 of chemotherapy at a fixed dose of 300 μg subcutaneously (S/C) every 12 h. All pts in the plerixafor group ($n=27$) received G-CSF at a fixed dose of 300 μg S/C every 12 h daily for 4 days. On day 5, if peripheral blood CD34+ was ≥20/μl, apheresis was started immediately. Plerixafor (240 μg/kg) was given 7-11 h before the first apheresis if CD34+ cell count on peripheral blood on day 5 was <20/μl and before the second apheresis if CD34+ cells on the first collect were <3ч10^6/kg. The median number of prior therapies was 1 (range 1-3) in both groups. The 2 groups were not different in terms of MM subtype, disease stage, and cytogenetic abnormalities. Table 1 shows the initial characteristics of pts.
Results: Compared with plerixafor, CY use was associated with higher median peak peripheral blood CD34+ counts (35 vs 111 cells/μl, $P=0.000003$), and total CD34+ cell yield (7.5ч10^6 vs 15.9ч10^6 cells/kg, $P=0.003$). All pts collected ≥4x10^6 CD34+ cells/Kg. Moreover, 60(96.7%) and 46(74.2%) pts in the CY group vs 24(88.8%) and 6(22%) pts in the plerixafor group collected >6ч10^6 and >10x10^6 CD34$^+$ cells/kg, respectively ($P=0.16$; $P<0.00001$). Only 4(6.4%) pts required 2 apheresis sessions in the CY group compared to 11(40%) in the plerixafor group ($P=0.0001$). Conversely, CY use was associated with more febrile neutropenia (60% vs 0%; $P<0.00001$), blood transfusions (27% vs 0%; $P<0.00001$), platelets transfusion (25% vs 0%; $P<0.00001$) and hospitalizations (64% vs 0%; $P<0.00001$). No one required intensive level of care and all recovered. Autografting was successfully performed in all pts using high-dose melphalan with a median time from mobilization to the first transplant of 31 days (range: 16-156) in the CY group compared to 13 days (range: 8-40) in the plerixafor group ($P=0.027$); and median infused CD34+ cells were 7ч10^6/kg (3.1-15.3) versus 5.27 (2.6-7.45), respectively ($P=0.002$). The average total cost of mobilization using the adjusted costs based on National Social Security Fund prices in Lebanon was slightly higher in the plerixafor group ($7964 vs $7536; $P=0.16$).
Conclusion: Our data indicate robust stem cell mobilization in MM pts with either fractionated high-dose CY and G-CSF or G-CSF alone with preemptive plerixafor. The chemo-mobilization approach was associated with two-fold stem cell yield, slightly lower cost but significantly increased toxicity.
Disclosure of Interest: None declared.

[P428]

Variables	Cyclophosphamide arm (N=62)	Plerixafor arm (N=27)	P-value
Male/Female	37/25	18/9	0.57
Age at transplant, median (range) years	55.5 (37-75)	54(35-66)	0.14
Serum creatinine >2.0 mg/dL	9	4	0.72
Disease phase at transplant			
First line	54	18	0.002
More advanced	8	9	0.036
Induction therapy			
Vincristin/Adriamycin/dex	25	0	0.00004
BTD/dex	15	8	0.84
Thalidomide/dex	10	0	0.03
BTD/Thalidomide/dex	10	4	0.77
BTD/lenalidomide/dex	1	12	0.000
Others	1	3	0.1
Disease status prior to autologous HSCT^			
CR	8	1	0.15
VGPR	10	8	0.19
PR	44	18	0.58

^International Myeloma Working Group Uniform Response Criteria; dex: dexamethasone, BTD: Bortezomib

P429

Biosimilar filgrastim (Leucostim®) seems to have similar efficacy in hematopoietic progenitor cell mobilization compared to original filgrastim (Neupogen®) and lenograstim (Granocyte®): a retrospective, multicenter analysis

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Introduction: Adequate hematopoietic progenitor cell mobilization (HPCM) is prerequisite for successful autologous hematopoietic cell transplantation (AHCT). Most centers use granulocyte-colony stimulating factor (G-CSF) alone as single agent for first step mobilization. Although we have lots of experience with filgrastim (Neupogen®) and lenograstim (Granocyte®) in terms of HPCM, data on biosimilarfilgrastim based HPCM is still scarce.Here we report our retrospective, multicenter analysis dealing with patients, who were mobilized with G-CSF alone using 3 different agents.
Materials (or patients) and methods: Medical records of patients who undergone HPCM with G-CSF alone, were retrospectively analyzed. Mobilization failure was defined as failure to achieve 0.5×10^6/kg, 0.8×10^6/kg and 2×10^6/kg $CD34^+$ cells in first, second and fourth days of apheresis, respectively.
Results: A total of 146 patients (female (n: 62); male (n: 84)), who were candidates for AHCT, participated in the study. Median age of study cohort was 50 (6-81). Indications for AHCT were Hodgkin's lymphoma (n: 22; 15%), non-Hodgkin's lymphoma (n:40; 27%) and multiple myeloma (n:84; 58%). Seventy-one, 32 and 43 patients received Neupogen®, Granocyte® and Leucostim®, respectively. Patients, who were treated with different types of G-CSF, were similar in terms of gender, primary diagnosis, and various risk factors for mobilization failure (age > 60, previous radiotherapy to bone marrow containing sites, bone morrow infiltration or fibrosis, previous exposure to lenalidomide or alkylating agents, pre-HPCM platelet count < 100000/mm^3 or high LDH).Median age of patients on Leucostim® arm (56; range: 19-81) were significantly higher compared to Neupogen®(50; range:6-71) and Granulocyte®(49; range: 16-65) arms;$P <$ 0.05).Following HPCM total number of collected $CD34^+$ cells were 18.3×10^6/kg, 11.07×10^6/kg and 12.66×10^6/kg, who received Neupogen®, Granocyte® and Leucostim®, respectively. There was no statistically significant difference between 3 types of G-CSF in term of progenitor cell yield ($P > 0.5$). Although there was a trend for decreased rate of mobilization failure in Neupogen arm (11.3%), treatment arms were similar in terms of mobilization failure (Granocyte 21.9% > Leucostim 18.8%; $P > 0.5$).
Conclusion: Although median age of patients who received Leucostim® was older than others who were on Neupogen® or Granocyte® arms, treatment groups were similar in term of established risk factors for mobilization failure.Biosimilarfilgrastim (Leucostim®) seems to be equally effective in HPCM compared to original filgrastim (Neupogen®) and lenograstim (Granocyte®), when used alone in first step mobilization.
Disclosure of Interest: None declared.

P430

Peripheral blood stem cell apheresis in small children

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Introduction: In low-weight children with cancer and healthy donor children, peripheral blood progenitor cells (PBPCs) have largely replaced bone marrow as source of autologous and allogeneic stem cells in part because of their relatively easy collection. However, there is a concern regarding medical, psychosocial and technical difficulties in small children.
Materials (or patients) and methods: We retrospectively analyzed peripheral blood stem cell apheresis in 24collections. Twenty patients were with cancer (11 patients = Neuroblastoma, 4 patients = Retinoblastoma, 3 patients = Germ cell tumor, 1 patient = Hepatoblstoma, 1patient = Wilm's tumor) and 4 healthy children donors. The study was conducted between 2012 and 2014. Peripheral stem cell apheresis was performed in the Mahak cancer children's hospital in a nice room for children where the patients stayed with their families. Patients were not routinely sedated. PBPC were collected by a COBE Spectra cell separator (COBE, Denver, CO, USA). Harvesting was performed after 5days mobilization.
Results: Mean body weight was 12.6 kg (range 8.9 kg–15 kg) for a median age of 3.3 years (range 1.1–5 years). Mean duration of harvesting was 210 min (range 174–274 min).Mean volume of stem cell collection was 145 ml (rang 120 ml - 250 ml). The mean number of total nucleated cells collected was 5.8 x10^8/ kg (range 3.3–8.9 x 10^8 /kg recipients). No side effects occurred. Children didn't require an additional haematopoietic progenitor mobilization or additional apheresis in other day. PBSC collection was without transfusion in healthy donor children.
Conclusion: PBSC collection may be difficult in small children owing to the large volume apheresis compared to the child's weight. Various problems, such as metabolic or haemodynamic disorders may be were seen. Peripheral Stem cell harvest can be performed in low-weight children under safe and effective conditions even when systematic priming by blood is avoided. Processing with increase of blood volume may to increase in the yield by recruiting progenitor cells.
Disclosure of Interest: None declared.

P431

Analysis of quality in 74 autologous hematopoietic progenitor cell products after cryopreservation: a significant influence of dilution matrix on viability and recovery of CD34+ cells

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Introduction: Harvest and transplantation of autologous hematopoietic progenitor cells (HPC) is a therapeutic option after high dose therapy for patients with different malignancies. Especially the quality of HPC products after cryopreservation is a decisive factor for successful engraftment. Here, we analyzed the influence of the dilution matrix after cryopreservation on viability and recovery of cells.
Materials (or patients) and methods: Viability of CD45+ and of CD34+ cells and recovery of CD34+ cells were analyzed in satellite tubes of 74 autologous HPC products after cryopreservation and thawing. Products were generated from 38 aphereses of 29 patients (10 female, 19 male) with multiple myeloma ($n = 20$) or with nonHodgkin's lymphoma ($n = 9$). Cells were thawed at +37 °C and rapidly diluted (1:10) in parallel in two different matrices, i.e. phosphate buffered saline with 10% AB serum (PBS) or Iscove's Modified Dulbecco's Medium without phenol red (IMDM). Cells were analyzed by FACS after shortened antibody staining but without lysis as published (Humpe et al., Transfusion,2005: 1208).
Results: The median viability of CD45+ cells after thawing was 68.0% (range: 34.5–89.5%) after dilution in PBS and significantly higher ($P < 0.001$) with 73.3% (range: 31.0–90.8%) after dilution in IMDM. With dilution in PBS, the median viability of CD34+ cells after thawing was 82.6% (range: 16.3–96.9%) and this was significantly higher ($P < 0.001$) at 93.5% (range: 29.8–98.8%) after dilution in IMDM. In addition, the median recovery of CD34+ cells of cryopreserved products,

defined as ratio between the number of CD34+ cells in the product after cryopreservation and the number of CD34+ cells in the product before cryopreservation, was significantly ($P<0.001$) higher with 90.8% in the IMDM group compared with 83.5% in the PBS group.
Conclusion: The presented results clearly show that a careful validation of quality control (QC) procedures for cellular products is mandatory. Especially as parameters deduced from such measurements are decisive for the final release of a HPC graft. In addition, the aim of such QC after cryopreservation should resemble the situation of the transplantation as close as possible. Since the patient's blood serving as the best dilution matrix is not available and suitable for FACS QC after cryopreservation the *in-vitro* matrix should be optimized. As result of these investigations the matrix used in our laboratory for QC after cryopreservation will be changed from PBS to IMDM.
References: Humpe A, Beck C, Schoch R, et al. Establishment and optimization of a flowcytometric method for evaluation of viability of CD34+ cells after cryopreservation and comparison with trypan blue exclusion staining. Transfusion ***45***: 1208–1213, (2005).
Disclosure of Interest: None declared.

P432
Use of the COBE 2991 to Wash Thawed Cord Blood Units

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Introduction: Post-thaw dilution and/or wash is recommended in the current FACT standards for all cryopreserved CBU and is mandated for all RBC-replete CBU. Although instruments are available to automate this process, CB washing is generally performed using a relatively manual technique whereby the thawed CB is diluted and transferred into blood transfer packs and centrifuged then the supernatant is removed using plasma extractor. This study was performed to determine whether the COBE2991 cell processor could be used to wash thawed CBU.
Materials (or patients) and methods: CBU rejected from routine banking by the Sydney Cord Blood Bank were thawed, diluted in 2.5% Dextran-40 and 2.5% human albumin in saline (DAS), centrifuged in the COBE2991 at 2200–3000 rpm for 5 minutes, then the supernatant was expressed and the cells resuspended in DAS. Cell recovery was compared to historical clinical data from CBU that were washed using the standard manual centrifugation technique prior to infusion.
Results:

	COBE2991 method	*Centrifuge method*	P #
Number of CBU [% RBC replete]	6 [50%]	12 [0%]	
Thawed volume (mL)	91 (49–133)*	42 (20–56)	0.0002
Thawed nucleated cells (x10^7)	86 (53–133)	116 (46–196)	ns
Thawed viable CD34 (x10^6)	1.0 (0.6–1.9)	3.3 (0.4–7.8)	0.0047
Thawed RBC (mL)	30 (17–46)	21 (5–25)	0.0076
Post-wash product volume (mL)	61 (49–64)	47 (18–65)	0.0232
Post-wash nucleated cells (% of thaw)	92 (82–109)	99 (86–121)	ns
Post-wash viable CD34 (% of thaw)	85 (67–121)	101 (65–135)	ns
Post-wash RBC (% of thaw)	45 (39–63)	47 (34–70)	ns

* Median & (range)
#Mann Whitney test

Conclusion: The COBE2991 yielded similar post-wash nucleated cell, viable CD34 and RBC recoveries to the historical data, despite the inferior quality of the CB used for COBE2991 study (low CD34 content and 50% RBC-replete). Benefits of the COBE2991 technique include greater automation and reduced time to perform the procedure.
Disclosure of Interest: None declared.

P433
The Sepax®2 or COBE®2991 cell processor for pre-transplant bone marrow processing – a comparison

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Introduction: In ABO-incompatible (ABOi) bone marrow (BM) transplantation (Tx), RBC and/or plasma is reduced in the harvested BM pre-Tx to avoid hemolytic post-Tx reactions. The COBE®2991 has been used for this purpose. In 2013 the Sepax®2 was validated for processing of major and minor ABOi BM harvests and for volume reduction of BM prior to freezing of autologous stem cells.
Materials (or patients) and methods: The first validation was performed with a mixture of pooled blood donor buffy coats (BC), packed RBC and NaCl to simulate a BM harvest regarding volume [1073 mL (798-1341 mL)], cells [TNC, 107x10^8 (94-138x10^8); MNC, 62% (53-69%)] and hematocrit [33% (22-49%)]. Hereafter, five BM harvests were processed to reduce incompatible RBC (double BC) and five BM underwent plasma reduction. The BM volume was 1068 mL (489-1571 mL), LPK was 185x10^8 (66-306x10^8), MNC was 71% (57-78%), and the hematocrit was 34% (25-53%). The Sepax®2 settings were adjusted during the validation.
RBC reduction. Step 1: the BM was first reduced in Sepax®2 using the program BM SmartRedux. The program settings for BC1 were: proportional volume, 30%; and reprocessed volume, 10 mL. Step 2: if the BC1 volume was >60 mL, BC1 was diluted 1:2 with 2.5% HSA in NaCl, and processed a second time in Sepax®2. Program settings for BC2: fixed volume, 60 mL; reprocessed volume, 0 mL. If the BC1 was <60 mL, the process continued with step 3. Step 3: the BC2 (or 1) was diluted 1:2 with 2.5% HSA in NaCl and thereafter pooled with one unit washed and packed ABO compatible RBC. The Sepax®2 was set to a fixed volume of 60 mL. Samples were taken at every step, and the remaining volume of incompatible RBC in the final product was calculated.
Plasma reduction. The BM was plasma reduced in Sepax®2 using the program BM SmartRedux. The program settings were: proportional volume, 100%; additional plasma, 0 mL; reprocessed BC, 0 mL; and reprocessed plasma, 0 mL. After processing, the BC was diluted with 2.5% HSA in NaCl to a hematocrit of ~40%. Samples were taken at every step, and the remaining volume of incompatible plasma in the final product was calculated.
Results: ***RBC reduction.*** Median recovery of TNC and MNC in the Sepax®2 was 80% and 87% compared to 76% and 86% in the COBE®2991. Median recovery of CD34+ cells in Sepax®2 was 84%. The volume of incompatible RBC was similar, 10.6 (Sepax®2) and 11.2 (COBE®2991) mL.
Plasma reduction. Median recovery of TNC and MNC in the Sepax®2 was 94% and 95% compared to 89% for both in the COBE®2991. Median recovery of CD34+ cells in Sepax®2 was 95%. The volume of incompatible plasma was similar in both machines, 2.1 and 2.3 mL/BW, respectively.
Conclusion: Sepax®2 is a good replacement for COBE®2991 for BM processing in major and minor ABOi BMTx. Sepax®2 shows good cell recovery (TNC and MNC), while maintaining the same depletion rate of incompatible RBC and plasma. Sepax®2 is easy to handle and can process big (up to 3000 mL) volumes of BM. A big advantage is that the process is far more automated compared to the COBE®2991-based process. Since 2014 we use the Sepax®2 routinely for pre-Tx BM processing in our lab.
Disclosure of Interest: None declared.

P434

PBSC mobilisation in children with metastatic neuroblastoma treated on the high risk neuroblastoma protocol - a single centre experience over 8 years

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Introduction: Children treated for high risk neuroblastoma require intensive combination chemotherapy and radiotherapy treatment including high dose chemotherapy with stem cell rescue to achieve remission. There is no ideal time for PBSC collection in patients with metastatic neuroblastoma, as earlier collection increases the risk of tumour cell contamination and later collections may fail due to poor marrow reserve. We reviewed all patients retrospectively who were treated on the high risk neuroblastoma protocol & referred to the West of Scotland Clinical Apheresis unit from 2006 -2014 for autologous PBSC collection.

Materials (or patients) and methods: All children (defined as aged 1–17 years) referred for PBSC collection between 2006 and 2014 were included in the study. Case notes of 29 children were reviewed retrospectively for number of mobilisation episodes, number of apheresis procedures per episode time from mobilising chemotherapy to first day of collection, highest peripheral CD34 count, total CD34 dose and the final fate of each PBSC collection. Comparative data were also collected for all children treated for Ewing's sarcoma who were referred for autologous PBSC collection during the same period. Statistics were analysed using SSPS.

Results: There were 29 children included in the neuroblastoma group and 25 children in the Ewing's sarcoma group. Children underwent a median of 1 PBSC collection episode in each group (neuroblastoma group range 1-5, Ewing's sarcoma group range 1-2). However peripheral CD34 counts (μl^{-1}) were significantly lower in the neuroblastoma group (median 33, range 8-577) compared to the Ewing's sarcoma group (median 122, range 12-798; $P < 0.0005$). Total CD34 doses collected (x 10^6/kg) were also significantly lower in the neuroblastoma group (median 3.80, range 1.22–27) compared to the Ewing's group (median 9.38, range1.90–28.86; $P < 0.0005$). This is despite a higher number of apheresis collection procedures per collection episode in the neuroblastoma group (median of 2 per episode, range 1-3) compared to the Ewing's sarcoma group (median 1 per episode, range 1-2). 10 children in the neuroblastoma group required additional plerixafor support compared to 3 children in the Ewing's group. Ten PBSC collections were discarded due to a positive pre-collection bone marrow in the neuroblastoma group, necessitating further collection attempts.

Conclusion: Our experience highlights the difficulties faced in obtaining transplantable CD34 doses from children treated for high risk neuroblastoma. There were strikingly poorer mobilisation outcomes (in terms of apheresis days, peripheral CD34+ counts and CD34+ cell doses) in children treated for neuroblastoma than with the comparator group of children with Ewing's sarcoma. We are aware that some other UK / Republic of Ireland apheresis services have also raised concerns about frequent problems collecting autologous PBSC with current treatment protocols for high risk neuroblastoma.

References:

Disclosure of Interest: C. Betts: None declared, J. Sinclair: None declared, E. Farrell: None declared, A. Doig: None declared, S. Graham: None declared, J. Sastry: None declared, K. Douglas Conflict with: Dr K Douglas has received honoraria from Genzyme and Sanofi in connection with speaker work and Medical Advisory Board participation, related to plerixafor

P435

Abstract Withdrawn

P436

Depletion of TCR$\alpha\beta$+ T cells and CD19+ B cells using the Miltenyi CliniMACS Cell Separation System

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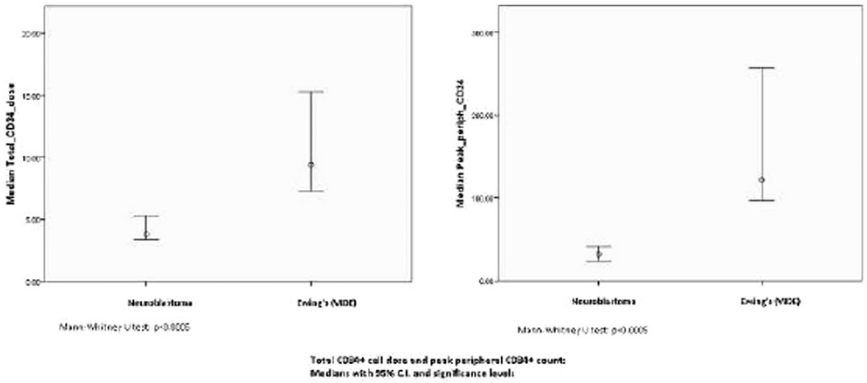
Introduction: T cell depletion of haemopoietic progenitor cell (HPC) collections has been demonstrated to reduce the incidence of acute and chronic graft versus host disease (GVHD) following allogeneic transplantation using alternative donors such as haploidentical or voluntary unrelated donors. Clinical systems for T cell depletion include the depletion of TCR$\alpha\beta$+ T cells and CD19+ B cells using the Miltenyi CliniMACS cell separation system. The increasing complexity of processing associated with the depletion of TCR$\alpha\beta$+ T cells and CD19+ B cells presents several challenges for the processing facility.

Materials (or patients) and methods: TCR$\alpha\beta$+ T cells and CD19+ B cells were depleted from HPC, Apheresis (HPC(A)) collected from 3 haploidentical donors and a matched unrelated donor using the Miltenyi CliniMACs cell separation system. The depletion was scheduled for day 0 of the transplant protocol with the procedure taking from 14 to 17 h to perform.

Results: The median number of CD34+ cells pre depletion was 45.8 x 10^6 /kg, TCR$\alpha\beta^+$ T cells was 15.9 x 10^8/kg and TCR$\gamma\delta$+ T cells was 3.6 x 10^7/kg. The median log depletion of TCR$\alpha\beta^+$ T cells was -4.1 (-3.9 to -4.3) and CD20+ B cells was -2.6 (-2.4 to -2.7). When >10 x 10^6 CD34+ cells per kg

[P434]



Total CD34+ cell dose and peak peripheral CD34+ count:
Medians with 95% CI, and significance level:

recipient weight were recovered, the infusion of TCRαβ+ / CD19+ depleted HPC(A) was limited to a maximum of 5 x 10^4 TCRαβ+ T cells per kg recipient weight ($n = 3$). All patients engrafted rapidly (ANC > 0.5 x 10^9/L between days 9–14 and Plt > 50 x 10^9/L between days 9–14) with minimal acute GVHD observed post transplant. One recipient of a haploidentical transplant using TCRαβ$^+$ & CD19$^+$ depleted HPC(A) developed EBV associated post transplant lymphoproliferative disorder.

Conclusion: Challenges for the processing laboratory include flow cytometric analysis for very low numbers of viable TCRαβ+ T cells and CD20+ B cells post selection that necessitates the analysis of large numbers of CD45+ cells (> 3 x 10^6 CD45+ cells) for a statistically significant result, the time taken to complete the procedure including the cryopreservation of excess HPC(A) collected but not depleted, and excess TCRαβ$^+$ T cell & CD19$^+$ B cell depleted HPC(A) not infused, and the maintenance of staff competency in an infrequent but complex processing procedure and the associated flow cytometric analyses.

Disclosure of Interest: None declared.

P437

Haploidentical Paternal TCRαβ and CD 19 Depleted Stem Cell Transplant For Severe Combined Immunodeficiency With Pneumocystis Jiroveci Pneumonia

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Introduction: In haploidentical hematopoietic stem cell transplant (HSCT), in vitro T-cell depletion of the graft is effective at preventing graft versus host disease (GVHD). Various T-cell depletion strategies have been used over time to minimize GVHD and maximize sustained engraftment and early immune reconstitution in alternative donor transplants with variable success rates. Depletion of T cell receptor alpha and beta (TCRαβ) and CD 19+ cells, with selection of TCRγδ T-cells is a new technique with promising results in haploidentical HSCT. We report a child with Severe Combined Immunodeficiency (SCID) with pneumocystis jiroveci pneumonia (PCP) treated successfully using this method.

Materials (or patients) and methods: Five months old male baby with SCID (T-B-NK+) with PCP requiring ventilator support was shifted to BLK SSH for further management. PCP was managed with septran and clindamycin. In the absence of suitably matched family or unrelated donor he was taken up for haploidentical paternal HSCT. He was conditioned using Fludarabine 30 mg/m2 (D-7 to D-3), Treosulfan 12gm/m2 (D-7 to D-5), Thymoglobulin 2.5 mg/kg (D-9 to D-6). GVHD prophylaxis included cyclosporine. Stem cells were mobilized using GCSF and peripheral blood stem cells were harvested. In vitro TCRαβ T cells were depleted using bioitnylated anti αβ antibody followed by antibiotin antibody conjugated to magnetic microbeads. B cells were depleted using CD19 conjugated microbeads. He received 8.5x10^8/kg total nucleated cells, 14.7x10^6/kg CD34 cells, 1.7x10^4/kg TCRαβ T cells (3.91 log reduction), 11.8x10^6/kg TCRγδ T cells, 3.7x10^4 CD19 cells (3.07 log reduction).

Results: Polymorphonuclear cell and platelet engraftment were seen on D+10 and D+12 respectively. Chimerism on day +15 showed 100% donor cells. There is no evidence of acute or chronic GVHD. He was discharged on D+28 in good clinical condition. He is currently D+75 post HSCT clinically well, on tapering doses of cyclosporine. His immune reconstitution assay shows he has good numbers of NK cells but T cells have yet not appeared.

Conclusion: Successful Haploidentical Paternal TCRαβ and CD19 Depleted Stem Cell Transplant for SCID despite PCP Infection. This appears to be a promising technique in haploidentical or MMUD HSCT with early and sustained engraftment, early immune recovery and less risk of GVHD. This is the first reported case of haploidentical HSCT using this technique for primary immunodeficiency in India.

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Disclosure of Interest: None declared.

P438

Bone marrow harvesting – bundle of preventive measures leads to negligible numbers of infectious contamination of harvested marrow

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Introduction: Despite efforts bone marrow harvesting is still associated with the risk of infectious contamination of harvested marrow. As centers usually do not share the data on actual infection frequency little is known on this topic. Our center is one of the biggest collection centers in Poland performing majority of the unrelated stem cell harvests in the country. Prompted by the occurrence of one case of infected bone marrow during the harvest at our center we incorporated a "bundle" of interventions directed at reduction of the procedure associated risk of contamination. Here we present results of evaluation of the method of bone marrow harvesting developed at our center.

Materials (or patients) and methods: The bone marrow harvesting in unrelated donors was done in semi closed methodology originally developed by Wiktor-Jędrzejczak & Pojda (1) for the first bone marrow transplantation in Poland in 1984. The method was further improved by replacing collecting bottles by bags for bone marrow collection. In 2012 a series of improvements ("bundle") to the original methodology was made that were directed at lowering risk of infectious contaminations. All steps of the procedure were analyzed by the harvesting team and the adjustments were made to improve all of them. Key steps of the procedure are i.a.: shortening of preparation of sterile materials in the operating room, limiting access to the operating room for personnel not directly involved in the procedure, two step skin decontamination (first, wash with antibacterial soap half an hour before the procedure and then second, scrubbing of the skin with antiseptic immediately prior the procedure in the operating room), securing of sterile operating field with transparent drapes, elimination of the first portion of harvested bone marrow to prevent possible contamination from the skin, reducing the open surfaces in the procedure to the minimum, intra harvesting NC check of the product and other.

Results: Since the last methodology improvement (September 2012) no overt bone marrow product contamination was seen in 114 conclusive harvests. There were 3 suspected marrow contaminations with Proprionibacterium acnes which were later not confirmed by control cultures at CC and cultures at TC. A movie with presentation of the methodology has been prepared for display at the EBMT conference.

Conclusion: While it is difficult to identify the exact cause of infectious contaminations of collected marrow every effort should be made to prevent its occurrence. We present here original rational approach and implementation of bundle of measures that lead to satisfactory results.

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A movie with presentation of the methodology has been prepared for display at the EBMT conference.

Disclosure of Interest: None declared.

P439

Situations affecting hematopoietic stem cell apheresis

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Introduction: In recent years,use of peripheral stem cell take place of use of bone marrow.To collect peripheral stem cell is cheap,painless and hematological recovery is faster.İn achievement of mobilisation, age,diagnosis, stage of disease, chemotherapy and radiotherapy, peripheral CD34 value is imprtant.In this study, we examined the patients who performed stem cell apheresis and planned present factors affecting results in order to contribute literature.

Materials (or patients) and methods: In our study,we retrospectively searched total 190apheresis data of 97patients who performed apheresis in Dokuz Eylül University Hematology Department,unit of apheresis betweenJune2012 and November 2014.Apheresis was performed by Fresenius comtec ve Spectra Optia devices.Pre-apheresis CD34counts by flowcytometry from peripheral blood sample of patients and donors was performed.If peripheral CD 34 count was higher than 10 μl,apheresis was performed to the patient.In this study,we examined demographic features, diagnosis, pretreatment CD34values,posttreatment mononuclear cell product CD34 results and product volüme, pre-apheresis complete blood counts of patients and donors who performed apheresis of peripheral stem cell, number of apheresis and devices which performed in apheresis.

Results: When total 190 apheresis of 97 patient were searched,we found that 38.9%of the patients were women and61,1%of thepatients weremen.Median age were 49,2.Diagnosis of patients were 42,1 multipl myeloma,22,1%nonhodgkinlymphoma,13,7%hodgkinlymphoma,9,5%AML,8,4%ALL and 4,2% others. Autolog transplantation was performed 80%of patients; allogeneic peripheral stem cell apheresis were performed 20%of patients.Mobilisation treatments were 26,8% G-CSF,cyclophosphamide plus G-CSF48,4%,4,7%prelixafor and 10%ESHAP and others.The devices used in apheresis were Fresenius comtec %33.7,Spectra Optia %66.7.Number of apheresis were mean 2,5 session(1-8),preapheresis CD34 value was mean 49.58μlproduct volume was mean 204,CD34 of product was mean 1411 μl,Plasma volume to colect was between 30-200 ml.We found statistically significant relation between entering WBC and CD34 value.In patients whose WBC were high, high product CD34 was collected. However, significant difference between entering thrombocyte and product CD34 was found($P = 0.004$).In patients whose platelet were high, product CD34 were also high. It's found that age and sex of patients didn't affect CD34 level. Significant difference between entering CD 34 and product CD34 values was assigned.While entering CD34 increase,CD 34 value was found high. In patients whose CD 34 was high, apheresis was achieved via less session.İn multipl myeloma patients, percent of product CD34 was significant high.-Difference between apheresis devices wasn'tfound.Product CD34 collected via Spectra Optia device was sinificantly high.However, when device and product volume were examine we found significant difference.It was determined that via spectra optia,less volüme but more CD34 product was collect.

Conclusion: Consequently, in this study,we discovered that for successful collection of product,diagnose of patient, pretreatment CD34 level,pretreatment WBC,platelet, devices used for apheresis ar important.The fact that via spectra optia,less volume but more CD34 product was collect evidenced our clinical observation.

Disclosure of Interest: None declared.

P440

Factors affecting the viability of CD34+ cells cryopreserved for autologous stem cell transplantation

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Introduction: Peripheral-blood mobilized hematopoietic Stem Cells (PBSC) are the primary source for autologous hematopoietic stem cell transplants (ASCT). PBSC harvested by leukapheresis (LK) are usually cryopreserved before ASCT. The major risk in cryopreservation lies in the freezing step, where PBSC may be damaged by formation of intracellular ice crystals. Thawing after cryopreservation is associated with a variable loss of viable CD34+ cells. Several factors (noncompliances during the freezing process, type and concentration of cryoprotectants, length of storage) have been suggested as potentially contributing to loss of viability; also, the degree of neutrophil (NEU) contamination in the harvest has been associated with a higher incidence of adverse events(1), but this aspect has never been examined in relation to the viability of CD34+ cells.

Materials (or patients) and methods: We performed a retrospective analysis to investigate the factors affecting the viability of freezed PBSC in 84 collections from 37 patients mobilized for ASCT for Acute Leukemia (AL), Lymphoma(LY), Multiple Myeloma (MM). The proportion of viable CD34+ cells was estimated with the ISHAGE single platform flow cytometric method (with 7-AAD exclusion), performed on a representative aliquot of the LK sample and was correlated with the following variables in a multivariate regression analysis (MVA): patient's age and diagnosis, viability before freezing, type of freezing (uncontrolled or controlled with the ICE-CUBE 14M® system), content of leukocytes (WBC), NEU and platelets (PLT) in a single bag (x 10^6/ml), haematocrit (HCT) of the LK.

Results: Indication for ASCT was MM, LY and AL in 41%, 43% and 16% of cases, respectively; 49% of patients was older than 60 years. At harvest, the mean viability was 99.7% (range 97-99.9%); controlled freezing was performed in 35% of LK. The median cell concentration of the LK was 225 x10^6/ml for WBC (range 69-419), 106 x10^6/ml for NEU (range 8-279), 445 x10^6/ml for PLT (range 30-3680); the median HCT was 1.2% (range 0.5-2.4). After diluting and splitting the harvest, the median content of CD34 in each bag was 85 x10e6 (range 8.4-481); the median concentration of NEU was 19 x10^6/ml (range 1.5-85), for PLT 76 x10^6/ml (5.6-1771).

As regard the viability of CD34+ cells, univariate analyses showed a marked difference between LK subjected to controlled and uncontrolled freezing (84% vs 54%, respectively, $P < 0.001$); a significant negative correlation was found between the final NEU concentration in the bag ($\rho = -0.39$, $P < 0.001$) and CD34 viability; the overall CD34 content of the bag was positively correlated with CD34 viability ($\rho = 0.24$, $P = 0.03$). The other variables examined did not result significantly associated with CD34 viability. In MVA, only type of freezing and the bag NEU concentration remained as independent factors associated with CD34 viability ($P < 0.001$ for both).

Conclusion: In this analysis we confirmed the superiority of controlled freezing to preserve the viability of PBSC cryopreserved for ASCT(2); moreover we observed a strong relationship between the concentration of NEU in the freezed bag and increased death of CD34+ cells: a possible explanation is that NEU release toxic lysosomal enzymes and oxidizing radicals during freezing and thawing, resulting in substantial toxicity to other cells.

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2. Montanari M et al, Transfusion 2003;43:42-9.

Disclosure of Interest: None declared.

P441

Does reinfusion of stem cell products in multiple days affect engraftment?

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Introduction: High dose melpahalan chemotherapy and autologous stem cell transplantation in multiple myeloma is still important treatment modality in transplant eligible patients. At least 2 x 10^6 /kg CD 34 dose is preferred for sufficient engraftment. Some patients need multiple leukapheresis procedures to reach required number of CD34+ cell but this can cause multiple transfusion packages that can not be given in single day. We want to evaluate the effect of reinfusion of stem cell in multiple days on engraftment results.
Materials (or patients) and methods: Demographic features, CD 34+ cell doses, neutrophile and platelet engraftment days, hospitalization days, number of infusion days of 85 multiple myeloma patients were evaluated retrospectively
Results: Median age was 56 ± 8.4 (34-68) with 40/45, M/F ratio. Mean CD 34+ cell number was 7.1 ± 5.3 x 10^6/kg. There was a significant negative correlation between neutrophile engraftment time and CD34+ cell number(11.2 ± 1.8 days). Mean platelet engraftment time was 13.0 ± 4.4 days. For 68 patients, reinfusion was performed in one day. But for 17 patients reinfusion was performed in more than one day because of high number of stem cell package. We didn't see any dimethyl sulfoxide toxicity, cardiac arythmia and volume overload complication. Hypertansive attack during infusion was easily controlled by frusemide infusion. In two groups (multiple infusion days (n:17) x single infusion day (n:68) mean CD 34+ cell levels were 4.1 ± 2.4 x 10^6/kg x 7.8 ± 5.4 x 10^6/kg. There were no statistical differences between two groups in case of platelet engraftment days; 15.18 ± 6.42 x 12.57 ± 3.7, $P = 0.12$) and neutrophile engraftment days respectively (12.18 ± 2.76 x 11.01 ± 1.55; $P = 0.11$). There was also no statistical difference between two groups in case of hospitalization days ($P = 0.7$).
Conclusion: In cases where more stem cell packages collected to obtain sufficient stem cell, reinfusion can be safely applied over several days without any delay in engraftment.
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Disclosure of Interest: None declared.

P442

Stem cell apheresis using the new Spectra Optia® CMNC platform

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Introduction: Granulocyte-colony-stimulating factor-mobilized peripheral blood stem cells, collected by white blood cell apheresis, are used for almost all autologous hematopoietic stem cell transplantations. Available manual apheresis systems generate stem cell products of consistently high quality. Continuous-flow and intermittent-flow blood cell separators (CFCS and IFCS) are both used to collect stem cells. Recently, Spectra Optia developed the Spectra Optia® IDL set for continuous CFCS. This study on a very small cohort of patients tried to determine the efficiency of this new Spectra Optia® platform.
Materials (or patients) and methods: Eleven (7 female and 4 male) patients with different hematological malignancies (NHL, MH and MM) were included in the study. Eight patients were mobilized after high dose chemotherapy (HDC) and application of non-glycosylated G-CSF. Three were mobilized with G-CSF only and one of them required Plerixafor. Four of the patients were considered "poor" mobilizers and were analyzed separately. They received additional growth factor one hour after initiation of the apheresis. One of the "poor" mobilizers had to undergo a second apheresis on the next day.
Results: The pre-harvest number of CD34+ cells in the "poor" mobilizers group was 30 ± 5/ μl (SEM), while the other 7 patients had a pre-harvest number of 421 ± 93,9/ μl CD34+ cells. The WBC was 38,6 ± 4,9x10^3/ μl. The pre-harvest platelet number was 41,5 ± 16,4x10^3/ μl. The total volume of processed blood was 8,09 ± 0,9 L. The final product volume was 164,4 ± 0,02 ml. The CD34+ cells yield was 8,75 ± 2,11x10^6/kg.BW. The collection efficiency (CE%) was 47,5 ± 4,6% for the poor mobilizers and 36,2 ± 5,6 for the good ones. The platelet loss was 16,7 ± 3,4%.
Conclusion: Collection efficiency does not differ significantly ($P > 0,1$) between "poor" and "good" mobilizers, indicating CMNC is performing in an equivalent manner across a range of pre-counts. The better CE% for the poor mobilizers' group may reflect the fact that they received 300 μg growth factor one hour after initiation of apheresis. The collection efficiency is lower than has been seen with the IFCS but this may reflect the fact that apheresis in the good mobilizers' group was terminated after the yield exceeded 10x10^6 CD34+ cells/kg.BW, as well as the small number of runs. The majority of pre-apheresis WBC were high where extraction efficiency can decrease but there are no indications of this happening in this small group of procedures. Increasing the collect flow rate to 1.2 for WBC > 20; 1.3 for WBC > 30 etc. might improve extraction efficiency. Platelet loss was low and this is advantageous for patients undergoing apheresis following mobilization with HDC.
Optia CMNC appears to be working reproducibly and acceptably within this small group of patient procedures across a wide range of starting conditions. CMNC acquired a CD34+ dose/kg > 2 x10^6/kg in 10/11 patients in one single apheresis and only one poorly mobilized patient required a second day of apheresis to achieve this dose.
Disclosure of Interest: I. Tonev: None declared, C. Botev: None declared, R. Smith Employee of: Terumo BCT, S. Hristova: None declared, Z. Mincheva: None declared, M. Mincheff: None declared.

P443

Efficiency of Peripheral Blood Progenitor Cell Collection in Allogeneic Donors Using the Spectra Optia® IDL Set in Comparison With the Spectra Optia® MNC Collection Set (ClinicalTrials.gov NCT01901458)

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Introduction: Peripheral blood progenitor cells (PBPC) are the preferred stem cell source for allogeneic transplantation. The most frequently used apheresis system was the Cobe Spectra (Terumo BCT), which will be discontinued shortly. Its successor - Spectra Optia (Terumo BCT)- provides two possibilities to collect PBPC: since 2010, the propagated "MNC" (mononuclear cell) set and software as well as the "cMNC" (continuous MNC) system, available in Germany since

late 2014. MNC combines continuous hard-spin centrifugation with subsequent cellular collection into a platelet elutriation chamber. PBPCs are harvested intermittently from this chamber. The cMNC system is based on a modified WBC-depletion procedure, which uses the IDL (intermediate density layer) disposable. With the IDL disposable it is possible to collect the target cells continuously using soft-spin centrifugation conditions. This investigator initiated trial presented here evaluated the performance and safety of the newly developed IDL based protocol in a prospective, dual center, open-label, randomized, study compared to the original MNC protocol/ disposable for the first time.

Materials (or patients) and methods: Between March and September 2014 fifty allogeneic donors were mobilized with glycosylated G-CSF (Lenograstim) at a total daily dose of 7.5 to 10 μg/kg BW donor, divided into two applications per day. Donors were randomized to either the MNC or IDL in a block wise fashion immediately before starting apheresis 2 h after the last morning G-CSF injection of day 5. MNC apheresis parameters were set according to manufacturer recommendations. In IDL collections modified WBC-depletion settings with low spin conditions and low collection pump flow rates were used. In donors, whose vein access permitted high inlet flow rates, heparin was used as an additional anticoagulant to citrate (ACDA). The performance was measured as collection efficiency (CE1, cells harvested/cells processed based on pre and post apheresis counts), throughput (TP1, PBPC harvested *10^6/kg BW donor/run time [h]/mean (pre and post) apheresis count [CD34 + cells/μl], and platelet loss. For results reported here, a significance level lower than $P = 0.01$ was chosen.

Results: Groups were well balanced for apheresis center, sex, age, weight, pre apheresis counts, and requested cell yield. 25 IDL and 24 of the MNC collections (one MNC drop out due to technical errors) could be analyzed for the performance parameters. Using the IDL set more CD34 + cells per unit time could be harvested (TP1 0.042 ± 0.009 compared to 0.026 ± 0.008 (MNC)) at comparable collection efficiencies (CE1 $74.3 \pm 13.7\%$ vs. MNC: $67.5 \pm 12.2\%$). Due to the intermittent collection in the "MNC"-mode there was a substantial loss of effective collection time (average calculated flow rate 52.4 ± 5.9 ml/min for MNC vs. 80.2 ± 14.1 ml/min for IDL). Although an additional elutriation platelet recovery process is implemented in the MNC procedure, much more platelets were lost compared to the IDL procedures (MNC: $41.7 \pm 14.9\%$ vs. IDL: 26.2 13 ± 11.4).

Conclusion: The IDL system used outperforms the MNC system in terms of PBPC harvesting velocity as well as in terms of platelet loss in the donor. These mobilized donors benefited from the shorter harvesting time, when the IDL platform was used instead of the MNC platform.

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P444

Comparison of four apheresis systems (Cobe Spectra, Spectra Optia MNC, Spectra Optia cMNC and Amicus) for non-stimulated mononuclear cell collections

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Introduction: Collection of mononuclear cells (MNC) as starting material for cellular therapeutics such as donor lymphocyte infusions, extracorporal photopheresis or dendritic cell vaccines is one of the main indications for leukapheresis - beside collection of peripheral blood hematopoietic progenitor cells. Moreover, diagnostic leukapheresi s might become a promising tool for the detection of circulating tumor cells in cancer patients.

The Cobe Spectra (Terumo BCT) was the most commonly used device for MNC apheresis in Germany. Because this device is discontinued, we compared it with three alternative apheresis systems for MNC collection in non-stimulated donors or patients, Amicus (Fresenius Healthcare), Spectra Optia MNC and Spectra Optia cMNC (both Terumo BCT).

Materials (or patients) and methods: 62 healthy donors and 116 cancer patients underwent a total of 268 non-stimulated MNC apheresis procedures within the last four years. Collections were performed using either Cobe Spectra (S, $n = 177$), Spectra Optia with MNC procedure (M, $n = 31$), Spectra Optia with cMNC procedure (C, $n = 20$), or Amicus (A, $n = 40$) according to the manufacturer's recommendations.

Results: Donor characteristics were similar between the four groups with respect to peripheral blood counts before apheresis. MNCs were most efficiently collected with the Spectra Optia cMNC system with a collection efficiency (CE1) of $64 \pm 11\%$ compared to $57 \pm 16\%$, $55 \pm 17\%$, and $52 \pm 16\%$ with Cobe Spectra, Spectra Optia MNC, and Amicus, respectively. With exception of the Amicus, the other devices harvested monocytes (CE1: $39 \pm 16\%$(A), $65 \pm 15\%$ (C), $58 \pm 19\%$(S), $61 \pm 21\%$(M)) more efficiently than lymphocytes (CE1: $57 \pm 19\%$ (A), $62 \pm 10\%$ (S), $56 \pm 17\%$ (C), $54 \pm 19\%$ (M)). Product purity in terms of percent MNC of WBC was significantly lower in products collected with the Cobe Spectra ($75 \pm 19\%$ versus $96 \pm 3\%$ (A), $92 \pm 7\%$ (M), or $90 \pm 7\%$ (C)). Hemoglobin (Hb) contamination of the products, analysed as CE1 (Hb) was low for both Spectra Optia procedures, highest for Amicus. Platelet CE1, which negatively influences the platelet contamination of the products, was significantly lower in Spectra Optia cMNC and Amicus compared to Cobe Spectra and Spectra Optia MNC.

The Spectra Optia cMNC allowed faster MNC collection compared to other systems: (MNC x 10^6 per minute) 69 ± 34 (C), 57 ± 22 (M), 56 ± 26 (S), and 53 ± 25 (A)).

Conclusion: Compared to the Cobe Spectra the current apheresis systems were superior in product purity and lower operator-dependance. The choice of an appropriate apheresis device for non-stimulated MNC collection depends on the intended product composition. Whereas Amicus procedures are suitable for platelet-sparing lymphocyte collections, the fastest and most effective collections of MNC and monocytes are performed by the Spectra Optia cMNC system.

Disclosure of Interest: None declared.

P445

CD34 + -selected stem cell boost in pediatric patients with poor grafts function after allogeneic hematopoietic cell transplantion (HCT), with either complete or uncomplete donor chimerism

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Introduction: The use of $CD34^+$-selected stem cell boost to improve poor engraftment function (PGF) after allogeneic transplantation is already known, however publications including pediatric patients are limited. Furthermore, some questions still remain uncertain, like the optimal number of these cells required, the effectiveness on patients with uncomplete donor chimerism, or the long-lasting quality of this therapy. We analyze the outcomes of three pediatric patients undergoing this procedure.

Materials (or patients) and methods: The poor graft function criteria was defined as hemoglobin < 85 g/L, leucocits $<$1x10E9/L or platelets $<$30x10E9/L, or higher levels if transfusions or growth factors were required to achieve these thresholds.

CD34 + -selection was performed by immunomagnetic separation using the CliniMACS Device® (Miltenyi Biotec) and no prior conditioning was administrated before the the boost infusion.

Results: Patient's characteristics:

	PATIENT 1	*PATIENT 2*	*PATIENT 3*
AGE (years)	13	9	10
DIAGNOSIS	T-cell lymphoproliferative disease (associated with EBV) + hemophagocytic lymphohistiocytosis	Severe aplastic anemia	Acute lympho-blastic leukemia (Ph +)
DONOR (HLA SIBLING)	RD (10/10)	URD (9/10; MM: B)	RD (9/10; MM: B)
SOURCE OF STEM CELLS	PERIPHERAL BLOOD	BONE MARROW	PERIPHERAL BLOOD
ABO AND Rh INCOMPATIBILITY	NO	NO	NO

RD: related donor; URD: unrelated donor; MM: mistmatch.

After allogeneic HCT patients 1, 2 and 3 achieved graft of neutrophils at days + 13, + 19 and + 23, respectively. All of them had complete donor chimerism within the first month from transplantation. However, they all presented cytopenias requiring red blood cells (RBC) and platelets transfusions, apart from erythropoietin and thrombopoietin.

In addition, patient 1 also needed support with G-CSF after the transplantation and could not discontinue until four months after the boost. This patient underwent the boost with uncomplete donor chimerism and was proposed to receive donor lymphocyte *infusions*.

Patient 3 was the only one to graft platelets at day + 153 from the transplantation and furthermore was the only one with reponse to thrombopoeitin. Unfortunately, three months later, he turned to lose the engraftment again.

The overage of $CD34^+$-selected stem cell boost infused was CD34 (x10E6/kg) was 4.3, 5.9 and 2.1, respectively; meanwhile, the dose of CD3/kg (x10E5) in the products was of 0.17, 0.17 and 0.03.

After the boost all patients presented an important increase in the hemoglobin and platelets levels and therefore the transfusion requirement were reduced. This was not so evident in erythrocytes transfusions for patient 3, who only required growth factors before the boost.

The overage of days for engraftment after the boost was of 69.3 for platelets and 69.6 for RBC. After the boost, days passed until platelet's engrafment were 110, 61 and 37. These dates for erythrocytes were 157, 5 and 47.

In relation to GVHD, only patient 3 presented stage 2 after de infusion of the boost. Neither death nor severe complications have been observed by now.

Conclusion: Our patients improved PGF after the infusion of the $CD34^+$-selected stem cell boost, without adding serious adverse events. Moreover, despite undergoing the boost without complete donor chimerism, patient 1 recovered from the pancytopenia.

Disclosure of Interest: None declared.

P446

Collection of Granulocytes for Transfusion to Neutropenic Patients: Performance Comparison of Two Apheresis Systems

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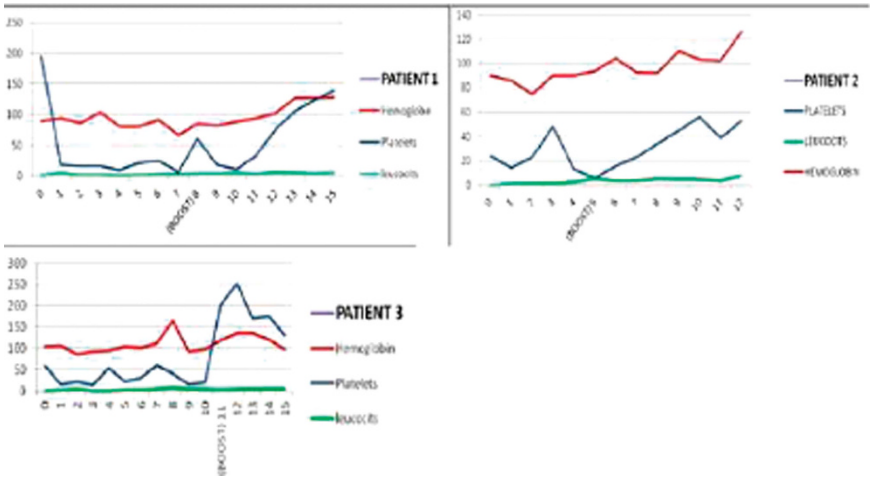
Introduction: Recently due to several reports of its efficacy, granulocyte transfusion has received renewed interest for treatment of neutropenic patients. Owing to modern apheresis devices and advances in biotechnology, sufficient and effective doses of granulocytes can be collected. In the current study we aimed to compare the ability of two different apheresis systems: Spectra Optia (Terumo BCT) and Com.Tec (Fresenius) to collect granulocytes.

Materials (or patients) and methods: 18-55 year old healthy volunteer donors were selected based on ABO major incompatiblity and free of illnesses and chronic medications that could interfere with an efficient donation. Donors received 2 GCSF injections (4 μg/kg) 16 and 8 hours before the procedure. Methylprednisolon (0,25 mg/kg) was given 14.8 and 4 hours before the apheresis started. To increase the sedimentation of red blood cells, 6% variHES (450kDa) was added but in a customized way: variHES was infused through site of the inlet line (50 drops/min) and ACDA 4% was added to blood through the dedicated line (13:1) for anticoagulation. Donors were randomly assigned to either Spectra Optia or Com.Tec system. Data are presented as mean ± SD. T-test and descriptive statistics were calculated with Statistica software.

Results: Granulocytes were collected from 48 donors (24 procedures/device) with no significant differences regarding age, gender, body weight and pre-procedure cell counts. (Table 1). Procedure time was significantly shorter on Spectra Optia. Similar blood volume processed on both devices but PMN Yield.[7.42 ± 2.84x1010 vs 5.05 ± 1.93x1010 granulocytes/

[P445]



product; $P < 0.01$] (Table 1) and therefore, CE% were both significantly higher on SO versus COM.TEC. Product volume collected with Spectra Optia was larger but purer: less RBC, platelet and MNC contaminations (Table 1).

Table 1	*Spectra Optia n = 24*	*Com.Tec n = 24*	*T-test P =*
Donor age (years)	29.00 ± 8.20	34.00 ± 9.33	0.055
Donor weight (Kg)	78.92 ± 15.91	82.67 ± 12.99	0.376
Donor PMN precount (10E9/L)	38.82 ± 7.81	37.10 ± 8.09	0.457
Blood vol processed (mL)	9867.62 ± 2101.84	9903.54 ± 1051.31	0.94
PMN prod (x10E10)- (%)	7.47 ± 2.84- 88.26 ± 7.02	5.05 ± 1.93 - 80.64 ± 10.14	1.21E-03 - 4.04E-03
Platelet prod (x10E11)	1.87 ± 0.48	7.22 ± 2.04	1.965E-16
Procedure time (min)	184.5 ± 17.32	226.08 ± 27.89	1.427E-07
Product vol (mL)	513.54 ± 57.29	391.33 ± 63.40	8.904E-09
Hemoglobin product (g/dL)	1.04 ± 0.39	4.93 ± 0.98	1.751E-22

Conclusion: Even if both system were capable of generating neutrophil concentrates, Spectra Optia system was more efficient and delivered a purer product in shorter procedure time. Adding High MW HES dropwise still does not seem to match the CE% we normally see in validated method but it shows it works – maybe it also minimizes the level of HES exposure to the donor.
Disclosure of Interest: None declared.

P447
Expiry date of the Cryopreservation Solution - a single-centre experience

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Introduction: Di-methyl Sulphoxide (DMSO) is commonly used as a cryoprotective agent for Peripheral Blood Stem Cells (PBSC) cryopreservation. A sterile 20%v/v solution of DMSO in 5.0%w/v Human Albumin Solution (HAS) is prepared under Good Manufacturing Pratice (GMP) conditions, stored at 2-8°C, and used subsequently as a medium for cryopreservation of human stem cells. Several centers only use this solution in the first 24 hours after its preparation. Could it be used for more than 24 hours?
Objective: To check whether the number of days between preparation and use (under sterile conditions) of DMSO solution affects the quality of cryopreserved cells. The cell's quality was assessed by cell viability, sterility testing and neutrophil recovery time (myeloid engraftment).
Materials (or patients) and methods: Under sterile conditions, in house prepared and refrigerated cryopreservation solution (20% v/v DMSO in 5%w/v HAS) is slowly added to the PBSCs or T-cells, at a 1:1 volume ratio, to a final concentration of 10% DMSO (for pediatric and clinically indicated patients final concentration is 5% DMSO). Products are frozen in a rate-controlled programmed freezer, stored between -150 and -196°C in vapor and/or liquid phase of liquid nitrogen containers and at the time of infusion are thawed at 37°C. A retrospective study was performed in 158 cellular products (PBSC and T-cell) cryopreserved in our lab between November 2011 and August 2014. The DMSO cryopreservation solution was used during a period of 0 to 40 days (median: 9 days) after preparation. Cell viability was evaluated by Trypan Blue method in 33 products (non-infused PBSC and T-cell of patients who died). The neutrophil recovery time (Absolute Neutrophil Count (ANC) > 0.5x10^9/L in 3 consecutive days) was measure in 125 patients infused with cryopreserved PBSC (minimum dose of 2x10^6CD34 + /Kg). All cryopreserved products were tested for microbiological contamination (mycological and bacterial (aerobic and anaerobic) cultures).
Results: It was observed that the number of days between preparation and use of DMSO solution did not affect neither cell viability nor neutrophil recovery time. Measured by *Pearson coefficient*, a very weak correlation was obtained between the variables (Cell viability, $R = -0.077$; Neutrophil recovery time, $R = -0.093$). Cell viability (%): 92.36 ± 5.04 [79 - 99]; N° days (d): 8.79 ± 7.06 [0 - 32]. Neutrophil recovery time (d): 11.40 ± 1.54 [8 - 22]; N° days (d): 11.37 ± 8.67 [0 - 40] (results presented as mean, SD, min – max). Cell viability maximum and minimum was 99% and 79% (PBSCs cryopreserved with solution prepared 13 and 16 days before, respectively). The maximum time between solution's use and preparation was 32 days (90% cell viability). Engraftment time maximum and minimum was 22 and 8 days (PBSCs cryopreserved with solution prepared and used on the same day and prepared 20 days before, respectively). The maximum time between solution's use and preparation was 40 days (ANC recovery in 10 days). The microbiological control of all products (158) was negative.
Conclusion: Cell viability and engraftment time are independent of the DMSO cryopreservation solution days (time between preparation and utilization). It is possible to prepare DMSO solution and use it up to 40 days.
Disclosure of Interest: None declared.

P448
Haploidentical T-cell depleted HSCT in children: cost analysis comparing 2 different T-cell depletion techniques

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Introduction: Haploidentical stem cell transplantation (haplo-HSCT) offers the possibility of an allogeneic transplant also to patients lacking a HLA-compatible family or unrelated donor. Moreover, the prompt availability of a haploidentical donor (usually the father or the mother) in the great majority of paediatric patients has the further advantage of making a timely transplant possible before a further relapse or disease progression. Different approaches have been developed over time, in order to overcome the HLA-barrier, to facilitate donor engraftment and to prevent acute and chronic GVHD. The use of T-cell depletion together with the infusion of stem cell megadoses is one of the most consolidated strategies. In recent years, a new technique of T-cell depletion, based on the physical removal of αβ+ T lymphocytes and CD19+ B lymphocytes (negative selection), was implemented, as an alternative to the classical positive selection of CD34+ cells.
Materials (or patients) and methods: We have retrospectively evaluated the cost of the T cell depletion procedure, the length of hospital stay and cost of hospitalization after HSCT, comparing 2 consecutive cohorts of children who received haploidentical T-cell depleted HSCT with positive CD34+ cell selection (12 patients) or with negative αβ+ T cell and CD19+ B cell selection (14 patients) in the period comprised from 2010 to 2014 in a single Italian pediatric HSCT unit. Both types of graft manipulation were performed using the CliniMACS device, Miltenyi Biotec GmbH.
Results: The cost of disposables and reagents necessary for the negative selection was higher than that of the positive selection [median 18,080 € per patient (range, 13,095-31,175) for negative selection and 13,095 € per patient (range, 6,026-24,107) for positive selection, respectively]. Two out of the 12 patients in the positive CD34+ cell selection group rejected the graft and were successfully re-transplanted with a different donor using the same T cell depletion technique, while all the 13 patients transplanted after negative αβ+ T cell and CD19+ B cell selection engrafted. Thus, on the whole, 14 positive CD34+ cell selections and 13 negative αβ+ T cell and CD19+ B cell selections were performed. The median length of hospitalization after HSCT was 60 days (range, 27-159) after positive CD34+ cell selection, while it was 29 days

(range, 17-80) after negative αβ+ T cell and CD19+ B cell selection. The calculated median cost of one day hospitalization in the HSCT unit, including overheads, diagnostic and lab expenses, medical supplies and drug provision, was 1,368 €, resulting in a median cost of 82,080 € (range, 36,936-217,512) for a transplant with positive CD34+ cell selection and a median cost of 39,672 € (range, 23,256-109,440) for a transplant with negative αβ+ T cell and CD19+ B cell selection.

Conclusion: Even though the cost of graft manipulation using negative αβ+ T cell and CD19+ B cell selection was higher than the cost of a classical CD34+ cell positive selection, the shorter duration of hospitalization after HSCT counterbalanced the higher cost of graft manipulation. Negative αβ+ T cell and CD19+ B cell selection could be cost effective as compared to other graft manipulation techniques in the context of haploidentical stem cell transplantation in children.

Disclosure of Interest: None declared.

P449

Correlation of pre-transplant CD34+ cell viability with apheresis concentrate parameters

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Introduction: Viability in cryopreserved hematopoietic progenitor cell-apheresis (HPC-A) is performed before transplant to ensure engraftment ability after infusion. Different methods may be used to evaluate HPC viability such as flow cytometry (7-aminoactinomycin D, 7-AAD, orange acridine, propidium iodide and monoclonal antibodies) and culture assays. We evaluated if the content of mature cells in HPC-A concentrates may influence CD34+ cell viability.

Materials (or patients) and methods: We evaluated HPC-A viability on satellite vials after freezing and cryopreservation by flow cytometry before infusion to the patients (pts). Cells with intermediate scatter between lymphocytes and monocytes, "bright" CD34 expression and "dim" CD45 expression were gated. An additional staining with 7-AAD permitted discrimination between viable and apoptotic cells. Viability was expressed as percentage of $CD34^+/7\text{-}AAD^-$ on total $CD34^+$ cells. We correlated $CD34^+$ viability with HPC-A concentrate parameters: white blood cells (WBC), neutrophils, hematocrit (Hct), platelets (PLTs), absolute $CD34^+$ counts.

Results: From January 2011 to September 2014 we performed 307 tests in 202 pts (NHL 85, MM 60, HL 24, solid tumors 18, AML 13, ALL 1, CML 1) with a median age of 53 years (range 1-67). All pts performed autologous HPC apheresis collection. All units were frozen within two hours from the end of collection with a controlled rate freezer. Viability tests were performed before transplant with a median interval from collection of 16 days (1-125): median viability was 94.8% (48.3-99.9). A statistically significant correlation was found between viability and concentrate PLT count ($P < 0.0001$): a lower platelet content was a better predictor of higher viability. Median PLT count was 0.43 $x10^6$/mL (0.05-4.05). A significant correlation was also found between viability and absolute CD34 content ($P = 0.014$).

Conclusion: These preliminary results can be useful to understand if the content of HPC-A units could have effect on $CD34^+$ viability and consequently on transplant engraftment. The effect of PLT count on HPC viability might be due to an interference with flow cytometry determination or reflecting a real reduction of HPC-A viability. In this last case, if confirmed, the reduction of PLT content (i.e. post-thawing washing) could have a positive effect on patients engraftment

Disclosure of Interest: None declared.

P450

Evaluation of Peripheral Blood Stem Cell Mobilization and Collection in Elderly Patients

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Introduction: Adequate hematopoietic stem cell mobilization and collection is essential for elderly patients who are candidate for autologous stem cell transplantation. In this study we compared mobilization success rates, amount of collected stem cells and the factors that could affect the procedure for patients younger and older than 60 years old.

Materials (or patients) and methods: For this study, 112 patients who admitted to Erciyes University BMT Center for autologous stem cell transplantation were enrolled. Thirty-three of them (36%) were under 60 years called young group and 76 of them (%64) over 60 years called elderly group. Among the participants, 73 of them were multiple myeloma, 23 of them Non-Hodgkin's lymphoma, 17 of them Hodgkin's lymphoma. Between the groups we compared the amount of pre-apheresis white blood cell (WBC), platelets, peripheral CD34+ cells, value of collected CD34+ cells and mononuclear cells, mobilization failure and success rates and number of apheresis sessions.

Results: The median values of pre-apheresis peripheral CD34+ cells were 8,72 /μl and platelets were 86 $\times 10^9$/L in young group; CD34+ cells were 8,95 /μl and platelets were 86,5 $\times 10^9$/L in elderly group ($P = 0{,}918$, $P = 0{,}899$). The median values of collected CD34+ cells were 7,61 $\times 10^6$/kg (2,52-46,62) and 7,60 $\times 10^6$/kg (2,87-25,50) in under and over 60 years, respectively ($P = 0{,}800$). Also the median values of total collected mononuclear cells (MNC) were 1,41 $\times 10^7$/kg and 1,4 $\times 10^7$/kg in young and elderly group ($P = 0{,}607$). It was found as 1,89 days in elderly group and 1,7 days in young group when we compared their apheresis sessions ($P = 0{,}786$). There was no statistically significance between two groups; despite the mobilization failure rates were 18% and 6% in patients older and younger than 60 years ($P = 0{,}087$). On the other hand, the number of multiple myeloma in the patients with applied autologous stem cell mobilization was higher in elderly patients than young ones ($P = 0{,}004$) and we also demonstrated that the failure of mobilization were lower in patients with multiple myeloma than lymphoma patients ($P = 0{,}003$). There was no significant difference between the amounts of pre-apheresis WBC, platelets and peripheral CD34+ cells in mobilization failure group and success group.

Conclusion: We demonstrated that the amount of pre-apheresis peripheral or collected CD34+ cells and numbers of apheresis sessions are not significantly different in comparison of the young and elderly patients who are planned autologous stem cells transplantation. Mobilization failure rate was higher in lymphoma patients than myeloma patients. It was also found that mobilization failure rates were higher in elderly patients than young patients.

Disclosure of Interest: None declared.

		Total	<60years	>60years	P value
Number of patients (%)		112	37(33)	75(67)	
Age	Mean		41	65	
Pre-apheresis	WBC (median)		8.95	8.72	0.918
	PLT (median)		86	86.5	0,899
	CD34+ Cell (μl)		8.72	8,95	0,918
Collected	CD34+ Cell (×10⁶/kg)		7,61	7,60	0,800
	MNC (×10⁷/kg)		1,41	1,40	0,607
Apheresis sessions (day)			1,7	1.89	0,607
Mobilization failure (%)			2(6)	13(18)	0,087

P451

Feasibility and advantages of a novel continuous Spectra-Optia apheresis system (cMNC-system) to collect non-stimulated mononuclear cells (MNC) for cellular therapy

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Introduction: Efficient and reliable apheresis procedures are required to collect non-stimulated mononuclear cells (MNC) from peripheral blood for therapeutic purposes, such as donor lymphocyte infusions (DLI). After introduction of the Spectra Optia® device MNC collections have been performed using the MNC-program employing an intermediate collection chamber from which platelets are elutriated from the buffy coat. In this single center pilot study we have investigated the feasibility of a novel continuous - Spectra Optia®- MNC (cMNC) device that was originally adapted from an intermediate intensity layer (IDL) white blood cell depletion procedure.
Materials (or patients) and methods: We prospectively analyzed in this single center pilot study our first non-stimulated apheresis procedures and DLI-products using the cMNC-apheresis system ($n = 17$) in comparison to apheresis performance and product composition after employing the Spectra-Optia-MNC settings with elutriation chamber ($n = 18$).
Results: Donor characteristics as well as pre-apheresis peripheral blood counts of T-, B- and NK-cells in both groups did not differ significantly from each other. More than all circulating blood lymphocytes (145% ± 63% cMNC vs. 155 ± 48%; MNC; n.s.) of the donors were collected into the product with both devices. Equal recruiting from lymphatic tissue back to peripheral blood during apheresis resulted in similar collection efficiencies (CE1) for all lymphocytes (60,0 ± 13,3 cMNC vs. 64,4 ± 14,8%; MNC; n.s.) as well as all subpopulations of T-, B,- and NK-cell. The MNC-purity in the products was high and comparable in both groups (85,3 ± 9,9% cMNC vs. 87,2 ± 3,5% MNC; n.s.). However, white blood cell (WBC) concentration in the DLI-product was significantly higher in the cMNC-group (100,3 ± 35,2/nl) compared to the MNC-group (76,9 ± 16,4/nl; $P < 0.05$). Although, final hematocrit was also higher in the cMNC-group (7,2 ± 1,7% vs. 4,9 ± 0,9% MNC; $P < 0.001$), this was only due to smaller total volume of the cMNC-product, and total RBC contamination in products was not significantly different between groups (12,8 ± 5,4 mL cMNC vs. 11,8 ± 3,6 mL MNC; n.s.). The total yield of 1,2 x 10^{10} lymphocytes in the final product did not differ in both groups and was comparable for all subpopulations.
The most striking differences between cMNC- and MNC-apheresis procedures have been detected in performance parameters. Significantly more blood was processed over a longer period of run time on MNC (14,6 ± 2,1 L) compared to cMNC (13,1 ± 2,7 L; $P < 0{,}05$) resulting in significantly larger volume products in the MNC group (238 ± 47 mL) vs. cMNC (176 ± 54 mL; $P < .005$) In addition, when the whole blood inlet flow was averaged over the procedure time, inlet flow rates were slower on MNC than cMNC resulting in slightly higher throughput (collected Ly/kg donor/minute/peripheral blood) in the CMNC group (508 ± 161) compared to MNC (421 ± 139; n.s.)
Conclusion: The novel Spectra-Optia®-cMNC apheresis system - that was originally adopted from the WBC-depletion set - allows faster and less donor demanding apheresis procedures with similar collection efficiency and product content for all donor lymphocyte populations. One additional benefit for the novel cMNC-system was the lower product volume compared to the MNC program.
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P452

Specific focusing on CD34 and CD3 viable cells from entire CD45 thawed population is more informative value for cryopreserved HSC and T cells recovery

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Introduction: The hematopoietic stem cells' dose for transplantation is usually based on fresh cells' enumeration. Analyzing the thawed product using the same strategy as used for fresh product usually ends with a higher percentage of CD34 cells and increased numbers of CD34/Kg compared to fresh results. The aim of this study was to specify the viable CD34+ cells' reservoir from the entire milieu of thawed product.
Materials (or patients) and methods: Enumerations were performed on fresh and thawed cells by using a dual platform according to the ISHAGE recommendations. Cells were stained for viability (using 7AAD), CD45, CD34, and or CD3. Analysis of the stained cells was performed by two strategies: 1. %CD34 positive cells from total viable cells (gated on CD45 positive and 7AAD negative population) and 2. Specific viable $CD34^{pos}$ or $CD3^{pos}$ subpopulations gated on CD45 positive cells. Results obtained from thawed samples ($n = 170$) were compared to their specific fresh origins, presented here as average ± SE.
Results: It was found that the average recovery of total WBC numbers was 104 ± 0.9% (range: 72-123%). The recovery of CD34 percent after thawing (calculated as %CD34 in thawed samples divided by the %CD34 in the original fresh sample) was 232 ± 80.1% (range: 84-379%). This higher percent of CD34 in the thawed samples calculated as higher absolute numbers of CD34 cells (x10^6/Kg) compared to their numbers in the fresh samples and the absolute numbers recovery is 132 ± 3.88% (range: 47.4-428.6). The recovery of CD3 percent after thawing was 134 ± 9.5% (range: 69-541%), which calculated as 113 ± 3.2% (range: 54-222%) recovery of their absolute population. Yet, measuring the specific $CD34^{pos}$ or $CD3^{pos}$ cells' viability in the total mixture of thawed cells without gating on viable cells only, indicated that their specific viability was 89 ± 0.83% and 87 ± 1.2%, respectively. These results correlate to the percent of viable cells analyzed by 7AAD from total thawed units (83 ± 1.0%).
Conclusion: Thus, we concluded that specific focusing on CD34 and CD3 viability is more informative value for cryopreserved HSC and T cells recovery.
Disclosure of Interest: None declared.

P453

Safety and efficacy of mobilized hematopoietic stem cell collection from peripheral blood in hematological patients and healthy donors - 14 years of experience

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Introduction: Mobilized hematopoietic peripheral blood stem cells PBSC become the preferred cell source for 99% autologous and 75% of allogeneic hematopoietic stem cell transplantation (SCT). Properly mobilized and harvested PBSC at the appropriate time before SCT is prerequisite for a successful transplantation. The collection of PBSC has become routine procedure worldwide. The aim of this study is to present our experience in collection of autologous and allogeneic PBSC in hematological patients and healthy donors in the last 14 years.

Materials (or patients) and methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of Macedonia and University Hematology Hospital between 2001 and 2015 in patients and healthy donors. PBSC harvesting was performed with continuous flow cell separator Baxter C53000 and COBE Spectra using conventional-volume apheresis processing. Mobilization regimens included granulocyte colony-stimulating factor (G-CSF) alone in healthy donors, and G-CSF alone or combination of G-CSF and disease-specific chemotherapy in patients. Minimum dose required to ensure successful and sustained engraftment was 2x10^6/kg CD34 + cells and 2x10^8/kg mono-nucleated cells (MNC).
Results: There were 585 apheresis procedures in total, of which 469 performed (80%) in 241 hematologic patients (152 males and 89 females, aged 18-65), and 116 procedures (20%) in 68 healthy sibling donors (45 males and 23 females, aged 20-54). Sufficient number of PBSC was collected with 2.0 apheresis in patients (range 1-5), and 1.7 apheresis in donors (range 1-3). The single procedure usually took 3-4 hours and the volume of collected stem cells was 50-220 ml. The tolerance of apheresis procedure in our patients and donors was good. The only adverse effects of the apheresis procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of anticoagulant (hypocalcemia), which occur rarely and were very mild. The main indications for autologous SCT in our patients were: multiple myeloma (35.7%), acute myeloid leukemia (29.6%), Hodgkin disease (17.3%), non-Hodgkin lymphoma (10.2%) and acute lymphoblastic leukemia (5.1%); and acute myeloid leukemia (56.6%), acute lymphoblastic leukemia (17.7%) and chronic myeloid leukemia (9.7%) in allogeneic SCT.
Conclusion: The collection of mobilized hematopoietic peripheral blood stem cells is an effective and safe procedure. Collection and transplantations of autologous PBSC makes 80% versus allogeneics (20%). All allogeneic donations were done by sibling donors. We should work on developing unrelated voluntary donation of stem cells. An adequate hematopoietic stem cell collection is fundamental for the success of the stem cell transplantation.
Disclosure of Interest: None declared.

P454
Single Centre Experience with the Continuous Mononuclear Cell Collection (CMNC) Protocol on the Spectra Optia® Apheresis System

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Introduction: Stem Cell collection procedures have evolved significantly over time with the most recent evolution being the Continuous Mononuclear Cell Collection (CMNC) protocol on the Spectra Optia® Apheresis System. At the Royal Adelaide Hospital prior to the introduction of the CMNC protocol in July 2014 we routinely used the Mononuclear Cell Collection protocol (MNC) on the Spectra Optia® Apheresis System.
Materials (or patients) and methods: For this study, we performed a retrospective data analysis on 40 Apheresis collections that utilised the CMNC protocol at the Royal Adelaide Hospital between July and December 2014. Our primary objectives were to assess collection efficiency, ease of collection and purity of Apheresis products. Our study included 25 patients (M 15, F 10) being mobilised for stem cell collection. The indications for mobilisation included 2 Allogeneic donors and 23 Autologous donors (7 Lymphoma, 15 Myeloma and 1 Sarcoma). Mobilisation regimes used for the collections studied were Cyclophosphamide/G-CSF (7 collections), Hyper CVAD/G-CSF (1), ICE/RICE/G-CSF (9) and G-CSF alone (23). All procedures were performed using the V11 CMNC protocol on the Spectra Optia® Apheresis System. When a literature search was performed on the CMNC procedure, there were no results found. This preliminary data may be the first on the CMNC protocol.
Results: All patients in this study group ultimately achieved a successful collection. The median pre-collection CD34/uL was 39.2 (7-466) and the median pre-white cell count 35.90x106/mL (4.0x106/mL-69.6x106/mL). The median collection efficiency for the CMNC protocol collections of 0.52 (0.29-1.14) compared favourably with apheresis collections performed using the MNC protocol on the Spectra Optia® Apheresis System with a median of 0.50 (0.08-1.36). The number of CD34x106/kg collected ranged from 0.45 to 27.39 with a median of 2.5x106/kg. Analysis of the apheresis product collected using the CMNC procedure, demonstrated an acceptable purity with a median granulocyte percentage of 39.5 (6-98) and platelet count of 739 x109/l (71-1813). The median white cell count of the Apheresis product was 133x106/mL (84-277) and the median volume was 268.0mLs (114-389mLs).
Conclusion: It is apparent from this study that the CMNC protocol was able to be used successfully over a wide range of indications, mobilisation regimes and pre-apheresis CD34 + counts. The protocol was user friendly enabling apheresis staff to fine tune the procedure for each patient. Our single centre retrospective study suggests that the CMNC protocol has a slightly improved collection efficiency compared to the previously used MNC protocol with no loss of purity of the product.
Disclosure of Interest: None declared.

P455
Assesment of thawed CD34 + viability predicts engraftment kynetics in Autologous Peripheral Blood Stem Cells (PBSC) transplantation

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Introduction: High dose chemotherapy followed by autologous haematopoietic stem cells (HSC) rescue is a standard of care in many haematological diseases. Flow cytometry assessment of CD34 + cells is generally accepted as a surrogate of the HSC content of the graft and therefore as an engraftment predictor. However, the viability of CD34 + cells at thawing is extremely variable and its impact on the engraftment has been poorly reported. We report here an analysis of 258 consecutive patients who underwent PBSC transplantation in our Institution.
Materials (or patients) and methods: Patients were diagnosed with either malignancies (246) or autoimmune diseases (12) and transplanted from January 2011 to June 2014 according to the local policy. All patients received intermediate/high intensity conditioning regimens. G-CSF was administered starting on day + 6 from HSC infusion. In case of double transplant, each procedure was analyzed separately. PBSC collection was aimed to reach a CD34 + cell count $\geq$2.5x106/Kg patient weight for each transplant procedure. Total and viable CD34 + cells were counted applying flow cytometric ISHAGE gating strategy in single platform before freezing and after thawing; viability was assessed by 7-AAD staining. PBSC grafts were cryopreserved according to the Center SOPs. Engraftment was defined as time to reach the first of three days with $0.5x10^9$/L Polymorphonuclear cells (PMN) and $20x10^9$/L Platelets (Plt).
Results: Count of Total Nucleated Cells (TNC) $x10^9$, Mononuclear Cells (MNC) percentage and viable CD34 + cells $x10^6$/Kg (mean ± SD) at freezing were 43.1 ± 32, 28.7 ± 15.2 and 6.2 ± 3.1, respectively. Two patients died before Plt engraftment at + 97 and + 128, respectively. All the others showed PMN and Plt engraftment (median and range) at 12 (8-46) and

13.5 (8-43) days from HSCT, respectively. Median viability of $CD34^+$ cells after thawing was 76.9 (4-99). The mean ± SD of viable $CD34^+ x10^6$/kg infused cells was 3.5 ± 2.3 (range 0.12-12.5). We arbitrarily considered the 10^{th} percentile of $CD34^+$ cells viability (25%) as a threshold. Patients who received a graft with a $CD34^+$ cell viability ≤25% showed a PMN and Plt engraftment kinetics slower than patients who had a graft with higher viability. Days to reach PMN engraftment (mean, CI) were 12.2, 11.9-12.5 vs 18.8, 15.3-22.3, $P = 0.001$. Days to Plt engraftment were 14.1, 13.6-14.5 vs 21.1, 17.6-24-6, $P = 0.001$.

Conclusion: Assessment of $CD34^+$ cells on thawed PBSC samples can provide an efficient quality control after freezing and can be a more valid predictor of engraftment kinetics than $CD34^+$ count before freezing. A more standardized technology for counting $CD34^+$ cells in thawed samples is still an unmet need

Disclosure of Interest: None declared.

P456

Performance reproducibility of the Spectra Optia device for HSC collection with the automated MNC Version7 program apheresis system

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Introduction: Spectra Optia cell separator is a widely utilized apheresis system for collecting peripheral blood stem cells (PBSC). Because of the automation of the procedure, few input were required from operator and we found interesting to explore if some parameters could potentially affect the product collected and the performance of the device.

Materials (or patients) and methods: In a single center experience, 15 patients (5 females and 10 males) were enrolled. A total of 29 autologous apheresis procedures were performed on Spectra Optia (program MNC version7.0, Terumo BCT). Different parameters related to patient, procedure, and product were collected and retrospectively analyzed. Procedure performance was evaluated on CD34 + cells collection efficiency (CD34 CE2) and calculated as 100 x (CD34 + cells/kg collected x body weight)/(CD34 + /μL x blood volume processed). Platelet loss was evaluated as 1- (platelet post count/platelet precount). Data are presented as median (min-max). Statistical analyses were performed using Statistica package.

Results: We observed a high correlation between the number of CD34 + cells circulating in blood and the total number of CD34 + cells collected/kg/L of blood processed ($R^2 = 0.92$, $P < 0.0001$) allowing a good prediction of blood volume to be processed to get the targeted CD34 + cell dose. Median CD34 CE2% was found satisfying (55% (19-140%)) and appeared to stay constant whatever CD34 ($R^2 = 0.013$;$P = 0.38$), white blood cells (WBC)($R^2 = 0.086$; $P = 0.16$), and platelet precounts ($R^2 = 0.006$; $P = 0.73$), as well as total blood volume (TBV) processed ($R^2 = 0.001$; $P = 0.40$). Product volume was primarily influenced by TBV processed ($R^2 = 0.52$, $P < 0.0001$). Circulating platelet and mononuclear cells (MNC) percentage in blood had only limited impact on final product volume per TBV processed ($R^2 = 0.12$, $P = 0.024$ and $R^2 = 0.29$; $P = 0.006$ respectively). It showed chamber on Optia was efficient in collecting mainly MNC and only few platelets. Platelet contamination of the product was found low (1.46x10E11 (0.82-4.98x10E11)) and seemed to be mainly influenced by the platelet precount ($R^2 = 0.67$, $P < 0.001$). Platelet loss on Optia was really low 15(-5-49) % and despite sometimes low platelet precount (94x10E9/L (55-295x10E9/L)), none of our patient needed any platelet transfusion after the procedure. Interestingly, platelet loss did not clearly correlate with platelet precount ($R^2 = 0.16$; $P = 0.065$) but appeared highly correlated with number of chambers collected ($R^2 = 0.56$; $P < 0.0001$) and in a limited way, with total blood volume processed ($R^2 = 0.2$; $P = 0.036$).

Conclusion: Optia was successful in collecting sufficient number of PBSC. The automatic mode allowed a high reproducibility of performance whatever procedure and patient conditions. We confirmed that the secondary separation on Optia (chamber) was efficient in extracting platelets from mononuclear cells and deliver a low platelet contaminated product as well as limit the platelet loss. To limit platelet contamination of the product and platelet loss, manual collect of chambers have to be avoided as much as possible.

Disclosure of Interest: None declared.

P457

Our experience of mobilization and collection of Peripheral Blood Stem cells for autologous transplantation in children with high risk Solid Tumors and Lymphomas

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Introduction: High dose chemotherapy followed by autologous hematopoietic stem cell transplantation (HSCT) has been recommended by actual treatment protocols for many high- risk childhood malignant tumors. Peripheral blood stem cell (PBSC) mobilization is usually performed following chemotherapy plus granulocyte colony-stimulating factor (G-CSF) in children. Various clinical and biological factors may affect stem cell mobilization and collection. Some important aspects such as optimal time to start collection, optimal pre apheresis CD 34 + cell count have not been still established in children. Our aim is to analyze our experiences with mobilization and collection of Ppheral Blood Stem cells for autologous transplantation in children with high risk Solid Tumors and Lymphomas in our clinic.

Materials (or patients) and methods: We retrospectively evaluated PBSC mobilization and collection records of 21 children who were scheduled to undergo autologous stem cell transplantation in our department in the 2010-2014 periods. G-CSF at the dose of 2x5 μcgr/kg/day was administered 24 hours after the disease specific chemotherapy protocol until the completion of PBSC. The aim was to collect at least $2x10^6$/kg CD34 + cells/kg. Data including age, sex, diagnosis, previous chemotherapy, number of mobilization, mobilization failure, previous collection attempts, use of plerixafor, number of apheresis, pre-apheresis CD 34 cell count, collection time, complications and collection outcome were analyzed.

Results: During study period, 22 children ((7 F/15M) underwent PBSC collection. While Nine of them had relapsed disease, the remaining had high risk solid tumor. Most patients were treated with different multiple chemotherapy regimens and underwent mobilization with the disease specific chemotherapy plus G-CSF. The diagnoses were relapsed Hodgkin lymphoma in four, high risk neuroblastoma in 14, recurrent Ewing sarcoma, Rhabdomyosarcoma, anaplastic Wilms tumor and Burkitt lymphoma in remaining 4 patients.The median number of mobilization attempt per case was one (range-1-3). The pre-apheresis CD 34 cell count ranged from 8 to 113/ μL, with a median of 28,5 μL. Juguler femoral or subclavian apheresis catheters were used harvesting in all cases(9,10,3 respectively). When CD 34 cell count was above 10/ μL, PBSC collection was performed on days 5-23 days with a median 11^{th} day.

The median yield of of CD 34 + cells was 2,87x10^6/kg per patient (range: 2,94-14,02). We used plerixafor in combination with G-CSF and chemotherapy in seven times in three patients with mobilization failure. Complications of PBSC mobilization and collection in our group were catheter-related thrombosis in two, catheter related infection in two and symptomatic hypocalcemia in two patients.

Conclusion: The success rate and tolerance of PBSC mobilization and collection was quietly good in our patients. There was no major complication related with procedure. Although the data regarding the use of plerixafor in children is scarce, our experience also supports its use in poor mobilizer children.

Disclosure of Interest: None declared.

P458

G-CSF primed donor haematopoietic stem cell collections are associated with reduced viable T cell yield for donor lymphocyte infusion

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Introduction: Donor Lymphocyte Infusions (DLI) are frequently used to enhance donor engraftment or treat relapse following allogeneic stem cell transplant. We aim to determine the factors that influence post-thaw viable CD3$^+$ recovery of cryopreserved donor lymphocyte collections at our institution from January 2006 to December 2013.

Materials (or patients) and methods: Donor lymphocytes were collected under 2 conditions: 1) Unprimed donor lymphocytes (CD3$^+$) at some time after transplant ($n = 38$). 2) G-CSF primed donor lymphocytes cryopreserved from the stem cell product after minimum infusion of 5.0x10^6/kg viable CD34$^+$ cells for transplant ($n = 35$). Factors analysed included viable CD45$^+$,CD3$^+$ and total nucleated cell (TNC), pre- and post-thaw recovery, donor sex/age, neutrophil and platelet content, cryopreserved nucleated cell concentration and the time interval between collection and freezing. Statistical analysis included parametric and non-parametric tests and two way analysis of variance.

Results: DLI harvests were obtained from 42 related donors and 31 unrelated donors. G-CSF primed collections contained a higher percentage of granulocytes, a lower number of platelets and were cryopreserved at a higher cell concentration. Although recovery of TNC was improved after thawing of G-CSF primed products (100 v 88%, $P < 0.001$), recovery of viable CD3$^+$ cells was reduced (62% for unprimed collections v 49% for G-CSF primed, $P < 0.001$; viable CD3$^+$ counts 76.8 v 43.1 x 10^6/kg respectively, $P < 0.001$). Multivariate analysis showed the factors significantly affecting viable CD3$^+$ recovery were: 1) harvest type: G-CSF primed compared to unprimed: $P < 0.001$; 2) Time to cryopreservation $P = 0.002$ for < 10 hours compared to 10-24 hours and > 24 hours.

Conclusion: Achieving adequate viable T cell doses for DLI infusions requires cryopreservation of over 10^8/kg CD3$^+$ cells. Our cell processing facility makes allowance for thawing losses of around 30% to 50% when freezing DLIs. Using G-CSF primed stem cell products and/or delaying time to cryopreservation can reduce viable T cell numbers upon thawing and may limit DLI doses available for infusion, particularly unrelated HPC collections from overseas. BMT Hospitals planning to administer multiple DLIs post-transplant may need to take these factors into consideration.

Disclosure of Interest: None declared.

Stem cell source

P459

Parameters that help predicting stem cell yield in pediatric transplantation

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Introduction: Hematopoietic stem cell transplantation (HSCT) has become an important therapeutic option for many malignant and nonmalignant disorders. However, there is significant interindividual variability in donors with regard to stem cell yield, target CD34$^+$ cell numbers could not be achieved in some donors. Recent studies analyzing the factors associated with stem cell yield were mostly performed in peripheral blood stem cells and exclusively in adult donors. Though, there is little information available concerning the factors that predict stem cell yield in pediatric donor population. In this retrospective study, we studied the factors associated with the bone marrow stem cell harvest yield in pediatric allogeneic transplantation.

Materials (or patients) and methods: Between April 2010 and November 2013, a total of 64 healthy related donors underwent bone marrow harvesting in our Bone Marrow Transplantation Unit. Donor demographic information, including age, sex, height, weight and body mass index was collected retrospectively. Steady state bone marrow was harvested from 35 donors. Bone marrow priming was performed in 29 donors with 10 μg/kg/day G-CSF for 3 consecutive days when the weight of donor and recipient was discordant.

Results: The median age of donors was 11.2 (range 1.2-50); 49 of them (76.6%) were less than 18 and 36 of them (56.3%) were less than 12 years of age. Linear regression analysis showed a strong negative correlation between CD34 + cell yield and donor's height (r = -0.643, $P = 0.0001$), donor's weight (r = -0.541, $P = 0.001$), and donor's age (r = -0.509, $P = 0.002$) in both donor groups who received G-CSF or not. In multivariate linear regression model analysis donor height showed the strongest effect on the CD34 + cell yield (model for body height, r = 0.626, $P = 0.0001$). Also, linear regression analysis in steady state bone marrow group revealed a strong positive correlation between platelet count (r = 0.608, $P = 0.0001$) and CD34 + cell yield. In the group who received G-CSF priming, there were strong positive correlation between pre G-CSF leukocyte count (r = 0.735, $P = 0.0001$), and pre G-CSF platelet count (r = 0.711, $P = 0.0001$) and CD34 + cell yield. Also, moderately positive correlation between pre G-CSF neutrophil count (r = 0.470, $P = 0.01$), and a weak positive correlation between pre G-CSF monocyte count (r = 0.364, $P = 0.05$) and CD34 + cell yield have been obtained. Multivariate regression analysis of this group revealed a persistent strong correlation between CD34 + cell yield and pre G-CSF leukocyte or pre G-CSF platelet counts. In our study, most valuable predictors for stem cell yield were donor's height and thrombocyte number. In ROC curve analysis, the cut off point for donor's thrombocyte number was 254x109/L [sensitivity:%76,4, specificity:%55,6, PPV: %91,6, AUC:0,727 (%95 CI: 0,555-0,900), $P = 0,01$] and the cut off point for donor's height was 154 cm [sensitivity: 72,7, specificity:88,9, PPV: %97,6, AUC:0,827 (%95CI:0,715-0,939), $P = 0,002$].

Conclusion: We concluded that the donor's height and thrombocyte number may be useful for predicting CD34 + cell yield before harvesting, especially when the size of donor was significantly smaller than the recipient.

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Disclosure of Interest: None declared.

P460
''On demand'' plerixafor as a mobilization regimen in multiple myeloma patients: a single centre experience using two different schedules

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Introduction: High-dose melphalan followed by single or tandem autologous bone marrow transplantation (ABMT) still represents a standard treatment for multiple myeloma (MM) patients in first remission. Successful peripheral blood stem cell (PBSC) harvest remains a key factor in ABMT, with a minimum number of 2.0 x10^6 CD34+ cells/kg required for a single procedure. Plerixafor (PLX), in association with G-CSF, is highly effective in increasing migration of PBSC into peripheral blood circulation both in proven and predicted poor mobilizer patients. Its use ''on demand'', ''just in time'' or as a pre-emptive therapy is highly recommended.
Materials (or patients) and methods: Thirty patients with MM (16 male and 14 female) were considered for mobilization with G-CSF plus PLX ''on demand''. Patients were divided into two groups based on the number of days of G-CSF administration: 13 of the 30 patients (group 1) received G-CSF for 4 days (PLX ''on demand'' at midnight of day +4), while 17 of the 30 patients (group 2) received G-CSF for 5 days (PLX ''on demand'' at midnight of day +5). Median age at the time of mobilization was 62.8 years (range 49.7–68.7) for group 1 and 66 years (range 50.8–70.9) for group 2, respectively. Median number of previous lines of chemotherapy induction was 1 (range 1-5) in group 1, and 1 (range 1-3) in group 2. Ten out of 13 patients (77%) in group 1 and 16 out of 17 patients (94%) in group 2 respectively received thalidomide as part of the induction therapy. At the time of mobilization no patients were in complete remission in group 1, while 3 patients obtained a complete remission in group 2; 11 and 13 patients were in partial or very good partial remission in group 1 and 2, respectively; 2 patients in group 1, and in 1 patient of group 2 stabledisease. A count of >20/μl CD34+ cells in peripheral blood was considered adequate to start leucapheresis. The apheresis target was 4 x 10^6 CD34+ cells/Kg, the minimum harvest required to perform two procedures.
Results: Overall 28 out of 30 patients (93%) mobilized >20/μl CD34+ cells in peripheral blood - 12/13 (92%) patients in group 1 and 16/17 (94%) patients in group 2. Ten out of 13 patients (77%) in group 1 and 8 out of 17 patients (47%) in group 2 received PLX on day +4 or on day +5 respectively. Median number of PLX vials used was 1 (range 1-2) in both groups. Overall 24 out of 30 (80%) patients achieved a yield of 4 x 10^6 CD34+ cells/Kg: 9 out of 13 (69%) in group 1 (median number of CD34+ cells/10^6/Kg: 4.3 (range 5-8.7)), and 15 out of 17 (88%) in group 2 (median number of CD34+ cells/10^6/Kg: 5.0 (range 4.3-8.7)). The median number of apheresis procedures was 2 in both groups (range1-2 and 1-3 groups 1 and 2, respectively). No grade 3 or 4 adverse events were observed in any group.
Conclusion: Our data confirms that an ''on demand'' strategy of PLX administration is safe and effective. Considering that almost all patients in group 2 received thalidomide (94%) as part of their induction therapy and almost all of them obtained both an adequate mobilization (94%) and CD34+ cells harvest (88%), we suggest that administration on day +5 seems to be the most cost-effective strategy.
Disclosure of Interest: None declared.

P461
First in Poland Use of Wharton's Jelly-derived Mesenchymal Stem Cells in Therapy of Neurological Disorders - Preliminary results

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Introduction: Multipotent, mesenchymal stem cells (MSC) in the human body, MSC are located mainly in bone marrow, adipose tissue, muscle tissue, Wharton's jelly (WJ), cord blood and lungs. The important feature of MSC from third party donor is the possibility of therapeutic applications, because of low expression of major histocompatibility complex class I proteins, and very low of MHC class II proteins. It has been demonstrated that MSC do not induce immune response. It has been also proved that MSC, in vitro, have immunomodulatory effects on antigen presenting cells (APC), Natural Killer cells (NK) as well as T and B lymphocytes. MSC obtained from the WJ compared to MSC derived from other sources, have a higher proliferation potential. The reduced activity of pro-inflammatory cytokines, including interferon gamma and tumor necrosis factor alpha have also been observed. Today there are many clinical trials worldwide in neurological diseases based on the MSC and therefore the Polish Stem Cell Bank established in Poland medical experiment addressed to patients (pts) with neurological diseases without causative treatment.
Materials (or patients) and methods: MSC were derived from WJ from third party unrelated donors, processed, frozen in liquid nitrogen and administered, after approval of Bioethical Committee, immediately after thawing. MSC characteristics were confirmed with flow cytometric immunophenotyping. Also, bacterial contamination and endotoxin content were excluded.
We have applied MSC either intravenously to 5 patients or intrathecally to one patient (pt), all with neurologic disorders. First, a 23-year-old male, was diagnosed with type 1 of hereditary motor and sensory neuropathy with central nervous system involvement and rod-cone dystrophy. Second, a 12-year old girl, was diagnosed with pervasive developmental disorder (PDD), optic nerve damage and hearing loss. Third - a 2-year-old girl was diagnosed in Romania with cerebral palsy (CP). Fourth, an 8-year-old male, underwent therapy with MSC because of autism, sensorineural hearing loss and intellectual disability. Fifth, a 24-year-old male received MSC in therapy of amyotrophic lateral sclerosis (ALS) and a 6-year-old girl is currently treated for spinal muscular dystrophy type II. One pt has already completed therapy with 5 MSC injections in 1-2 month intervals, the rest have received from 1-3 doses. The mean dose equaled 1x10^6/kg recipient body weight/injection and the stem cell viability was >90%.
Results: No dangerous side effects were detected during MSC infusions. No adverse effects were noticed one month after infusions apart from transient tachycardia, transient headache or bone pain similar to pain in viral infection. There was no need of medical intervention. The first positive clinical symptoms were observed in all pts. However, the most important, notable fact was pts condition improvement. In girl with CP less presented muscle spasticity has been observed (after one month already), in girl with PDD the reduction of auto-aggression and in adult with ALS there was an improvement in muscle strength.
Conclusion: The intravenous infusion of third party donor WJ-derived MSC is a safe procedure with positive clinical effects.

Further studies are needed concerning number of injections and intervals between them as well as the optimal dose of MSC in each infusion.

References:

Disclosure of Interest: D. Boruczkowski Employee of: The Polish Stem Cell Bank, M. Chroscinska-Krawczyk: None declared, M. Murzyn Employee of: The Polish Stem Cell Bank, I. Czaplicka-Szmaus Employee of: The Polish Stem Cell Bank, A. Olkowicz Employee of: The Polish Stem Cell Bank, D. Gladysz Employee of: The Polish Stem Cell Bank, M. Boruczkowski: None declared, T. Oldak Employee of: The Polish Stem Cell Bank

P462

Decrerased transplant related mortality and older patients in 20-years autologous stem cell transplantation programm, single centre results

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Introduction: We present data from our transplant centre in Prague, which currently celebrates 20 years of autologous stem cell transplantation (ASCT) programm. The 1th ASCT was on december 1993. Untill now we performed 1389 ASCTs in 1112 patients, 147 tandem, 34 triplet transplants. The median age of patients was 56 years (18-71).

The main indications for ASCT were lymphomas: NHL 42% ($n = 586$, DLBCL = 267, FL = 124, and others), multiple myelomas (MM) 37% ($n = 509$) and Hodgkins lymphomas (HL) 10% ($n = 137$). Other diagnoses comprise breast carcinoma, multiple sclerosis, solid tumours and others.

Materials (or patients) and methods: Overall 267 patients with DLBCL underwent ASCT. Ten-years overall survival (OS) is 62% in the whole group. Even better survival curves we obtained comparing not-relapsing ($n = 187$) and relapsing ($n = 80$) patients after ASCT, with 10-year OS 84% and 22% respectively.

124 folicular lymphoma patiens were transplantated with 10-y OS 57% (PFS 54 months)

113 patients with Hodgkin lymphoma underwent ASCT with 10-years OS 54%. Relaps after ASCT ($n = 48$) was strong predictor of low OS (24 months).

361 MM patiens were transplanted with 10-years OS 38%.

Results: Trends in transplant acitivities:

Significant improvement in peritransplant complications was recorded in the course of the last two decades. Despite the increasing median age of patients (table), transplant related mortality is decreasing.

The table shows significant part of ASCTs in HL in the beginning of the programm has been moved for the benefit of MM and particulary aggressive lymphomas such as MCL.Table: Age, TRM and frequency of major diagnoses are changing in time:

	1994-2000	*2001-2009*	*2010-2014*
Age (median, years)	46	50	57
TRM ($\leq$ day $+100$)	7,2%	3,8%	2,7%
NHL	50,5%	54,7%	50%
HL	26%	8,3%	4,7%
MM	23,5%	37%	45,3%

ASCTs for breast cancer started in 1994 and 46 patients were enrolled untill 2000.

From 2001 to 2010 we have transplantet 26 patients with multiple sclerosis receiving BEAM conditioning with T-cell depletion.

Conclusion: Although indication criteria for ASCT are changing in time, ASCT still remains fundamental procedure in the treatment of lymphoma and myeloma patients.

The developing supportive measures in transplant standards last years offer ASCT to older patients with lower risk of transplant related mortality.

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Disclosure of Interest: None declared.

P463

Assessment of CD34+ cell viability at thaw/infusion for dual-unit cord blood transplantation: Correlation of viable CD34+ cell dose with engraftment

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Introduction: Viability of CD34+ cells upon thaw/infusion has recently become a significant marker of the quality of a cord blood unit (CBU). The aim of this study was the comparative analysis of CD34+ cell dose at cryopreservation and at thaw/infusion, and its association with engraftment in the context of double unit cord blood transplantation (dUCBT).

Materials (or patients) and methods: The study included 56 CBUs, that were thawed for infusion in 28 adult dUCBT recipients with haematologic malignancies, from 4/2009 to 9/2014. Upon thawing, the cryoprotective solution (DMSO 10%) was either removed by centrifugation/washing or diluted in a less hypertonic solution of Dextran 40/Human Albumin 2.5%. Enumeration of CD34+ cells was performed by single-platform flow cytometry, according to ISHAGE guidelines. CD34+ cell viability was evaluated by addition of 7-AAD dye and sequential Boolean gating strategy.

Results: There was a significant difference between total CD34+ cell counts at cryopreservation and viable CD34+ cell values at thaw (Wilcoxon test, $P < 10^{-4}$). Despite reduction post-thaw, the counts of viable CD34+ cells did correlate with the corresponding values at cryopreservation (Spearman's rho: 0.83, $P < 10^{-4}$). CD34+ cells retained high viability after thaw, with 91% of CBUs (51 out of 56 tested) demonstrating CD34+ viability $\geq$80%. Viability of $<$50% was noticed in only one CBU that failed to engraft. Viable CD34+ cell dose of the dominant unit was significantly associated with the cumulative incidence of engraftment (SHR: 2.39, 95% CI: 1.06-5.37, $P = 0.034$).

Conclusion: In conclusion, CD34+ cell viability at thaw/infusion is a relevant criterion of the adequacy of a CBU for allogeneic stem cell transplantation. Moreover, viable CD34+ cell dose of the prevailing unit is predictive of engraftment kinetics in dUCBT.

Disclosure of Interest: None declared.

P464

Impact of GCSF primed bone marrow on the outcome of allogeneic hematopoietic stem cell transplantation in adolescent and young adult with severe aplastic anemia

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Introduction: Steady state bone marrow (SS-BM) is the standard source of hematopoietic stem cell in allogeneic hematopoietic stem cell transplant (HSCT) from related sibling donors in patients with severe aplastic anemia (SAA). However, the use of G-CSF primed bone marrow (G-BM) as a stem cell source is usually limited and only considered in transplant practice in the presence of major ABO-incompatibility (requiring red cell depletion) or significant difference in the weight between the donor and recipient in an attempt to obtain adequate SC dose without increasing the risk of graft versus

host disease. Here, we report our center's experience of HSCT as the first cohort in adolescent and young adult patients with SAA using SS-BM or G-BM with long term follow up.

Materials (or patients) and methods: We retrospectively evaluated outcomes in adolescent and young adult patients who had an allogeneic HSCT for SAA from January 2002 -June 2014 with G-BM as stem cell source and compared it with same time cohort control group who received SS-BM.

Results: A total of 23 patients (study group) received the marrow graft primed with G-CSF 5 μg/Kg/day for 4 days before marrow harvest, and 59 patients (cohort control group) received marrow graft without G-CSF priming. Patient's characteristics were comparable in both groups and are shown in table 1, with a median follow up of 72 months (range, 24-115). All donors were related HLA-identical siblings except for two patients who had one antigen mismatched sibling donors. Conditioning regimen was CY/ATG from January 2002- June 2004 and FLU/ATG from July 2004-June 2014. All patients received the same graft-versus-host disease (GVHD) prophylaxis (Cyclosporine and Methotrexate).The median time to both neutrophil and platelet engraftment (ANC $> 0.5 \times 10^9$/L; platelet $> 20 \times 10^9$/L) were comparable in both group, at 21 versus 20 day and 25 versus 22 days in the G- BM and the SS-BM groups respectively. No graft failure was reported in the G-BM group but was observed in 8.5% of SS-BM group ($P = 0.1$). The incidence of grade II-IV acute GVHD was not statistically different, 13.6% in the G-BM group and 23.8% in the SS-BM group ($P = 0.1$); chronic GVHD was significantly lower in the G-BM group (5%) compared to SS-BM (26.3%) ($P = 0.04$); overall survival rates (91.3% versus 80.6%) between the primed and unprimed marrow group.

Conclusion: G-CSF mobilized bone marrow is a reasonable stem cell source in patients with SAA and Major ABO incompatibility or significant donor/recipient weight discrepancy, resulting in comparable engraftment with no reports of graft failure in this series and no increase in GVHD risk in comparison to SS-BM source. The above observation should be further tested in larger prospective trial as the optimal stem cell source in patient with severe aplastic anemia after HLA-identical related HSCT.

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Disclosure of Interest: None declared.

Table 1. *Patient Characteristics*

	Study Group	Control Group
No. of Patients	23	59
Age, median (range), y	19(16-26)	22(17-26)
Sex, F/M	14/9	23/36
Donor age, median (range), y	12(9-20)	24(17-29)
Donor sex, F/M	7/16	22/37
ABO match		
Compatible	9(39.1%)	42(71.2%)
Major	7(30.4%)	9(15.3%)
Minor	7(30.4%)	8(13.6%)
CD34 x 10^6/kg (median)	3.1(1.9-5.1)	3.15(2.18-4.54)

P465

Impact of stem cell dose and performance status on cord blood transplantation outcome in adult patients with advanced hematologic diseases: 13-year experience in Korea

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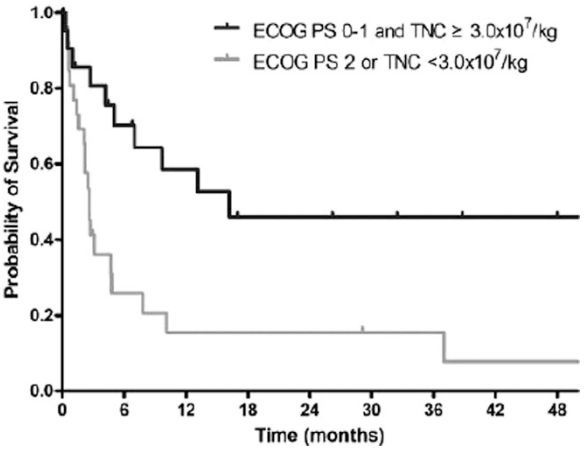
Introduction: Cord blood transplantation (CBT) is an established alternative donor transplantation for treatment of patients with advanced hematologic diseases. Nevertheless, CBT has not yet been performed frequently in adult patients in Korea because of high incidence of graft failure and non-relapse mortality (NRM).

Materials (or patients) and methods: To assess clinical outcome after CBT and provide future recommendations for alternative donor, we conducted the first retrospective multicenter study of adult CBT in Korea.

Results: Between June 2001 and April 2014, 48 patients (male, 19) were enrolled from 16 hospitals. The median age was 36 years (range, 16-65 years). Forty patients (83.3%) were acute leukemia: 33 (68.8%) in remission and 7 (14.6%) in persistence. The others were myelodysplastic syndrome ($n = 3$, 6.3%), severe aplastic anemia ($n = 3$, 6.3%), and lymphoma ($n = 2$, 4.2%). Thirty two patients (66.7%) underwent double unit CBT. Twenty two patients (45.8%) underwent myeloablative conditioning and 26 patients (54.2%), reduced-intensity conditioning. Total body irradiation (TBI)-based conditioning regimens were used in 23 patients (47.9%). The median time to neutrophil ($> 500/\mu L$) and platelet ($> 20{,}000/\mu L$) recovery among engrafted was 20 days and 35 days, respectively. Fourteen patients (29.2%) failed to achieve neutrophil $> 500/\mu L$, and 22 patients (45.8%) failed to achieve platelet $> 20{,}000/\mu L$. The 2-year overall survival (OS) was 29.6%. In multivariable analysis, ECOG performance status (PS) ≥ 2 (hazard ratio [HR] 6.42, $P = 0.001$) and total nucleated cell (TNC) dose $< 3.0 \times 10^7$/kg (HR 2.59, $P = 0.012$) were poor prognostic factors for OS (Figure). Factors associated with graft failure were PS 2 (83.3% vs. 19.2% at 100 days; $P = 0.012$) and non-total body irradiation-based conditioning (44.0% vs. 6.8% at 100 days; $P = 0.026$). CBT outcomes after January 2009 were significantly improved compared to those before January 2009 (2-year OS: 43.5% vs. 18.1%; $P = 0.012$). This improvement is due largely to higher TNC dose (median 4.30×10^7/kg vs. 2.61×10^7/kg) and better PS were included (PS 2: 9.1% vs. 15.4%) since 2009 compared to before 2009.



Conclusion: Although in the past, the clinical outcome of adult CBT was not satisfactory in Korea because of high NRM, it has been improved in recent years. Our data suggest that the clinical outcome substantially depends on the cord blood cell dose and PS. On the basis of this analysis, we are planning a prospective multicenter study of adult CBT in Korea.
Disclosure of Interest: None declared.

P466
Harvest of Bone Marrow grafts have deteriorated in recent years

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Introduction: To obtain a "good" stem-cell rich bone marrow (BM) harvest, an experienced physician with good aspiration technique is of outmost importance. As the number of BM aspirations have decreased dramatically during the last decades the logical consequence is that the opportunity to get this experience has decreased considerable. Furthermore, we wanted to investigate if there is any difference in quality (measured as relative content of CD34+ cells) between BM grafts aspirated at our center for our own patients and BM grafts acquired from another center where no relationship exist between physician and patient.
Materials (or patients) and methods: We analyzed the relative content of CD34+ cells in BM grafts given to patients undergoing HSCT at our center between 1995 and 2011. We also compared BM harvests performed at our center (sibling donors) with harvests performed at other centers (URD).
Total nucleated cell count and CD34+ cell count were analyzed. CD34 enumeration was performed using a single platform flow cytometry analysis based on ISHAGE gating strategy.
The relative CD34+ cell counts were compared with Mann-Whitney U test or Kruskal-Wallis test. Factors affecting the CD34+ cell yield were analyzed with multiple regression.
Results: A total of 256 BM grafts aspirated at our and other centers were analyzed and compared. The median (range) relative CD34+ cell content in these BM grafts were 1.07% (0.23-3.45). The material was divided into six three-year periods. In the first period (1995-97) the median CD34+ content was 1.57% compared to 1.01% in the last period, (2010-11)($P<0.001$). This may indicate a probable deterioration in the skill in aspirating BM during the last years. Median donor age was 30 years (1-63), 71 (27%) were younger than 20 years. According to our results grafts harvested from a donor younger than 20 years of age contains considerable higher yield of CD34+ cells, while the yield in older donors seems to be rather stable. For this reason we also studied the BM quality during the years only in donors aged older than 20 years. This analysis showed similar results as if all donors were included independent of age.
BM grafts aspirated for our own patients had significantly higher CD34+ content compared to grafts obtained from other centers ($P<0.001$). To verify these results we performed a multivariate analysis of factors affecting the CD34+ cell content. Factors with significant effect were: Year of harvest [RH 0.77, 95% CI 0.68-0.87, $P<0.001$], donor age [0.62, 0.54-0.70, $P<0.001$] and "own patient" [1.20, 1.06-1.37, $P=0.005$]. Restricting the multivariate analysis to donors older than 20 years of age showed similar results: Year of harvest [RH 0.73, 95% CI 0.62-0.85, $P<0.001$], donor age [0.79, 0.67-0.92, $P=0.004$] and "own patient" [1.20, 1.02-1.40, $P=0.026$].
Conclusion: These results may indicate that the skill in how to aspirate a BM graft of good quality has decreased over time. When aspirating BM, it is important to know that small volumes, 1 and no more than 3 ml, should be aspirated to get best possible yield. Physicians' active during the BM-graft intensive years (before 2000) probably still has the skill while younger physicians have less experience. As BM may increase in the future with more haploidentical transplants for e.g., it will be of importance to educate younger physicians in time in order to achieve adequate bone marrow grafts since the cell dose has significant impact on survival.
Disclosure of Interest: None declared.

P467
Haploidentical Stem Cell Transplantation (HAPLO-HSCT) With Post-Transplant Cyclophosphamide (PTCy) As GVHD Prophylaxis In High Risk Hematologic Malignancies: Bone Marrow or Peripheral Blood Progenitors Render Same Results

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Introduction: Allogeneic transplantation is the only curative option for patients with high risk hematologic malignancies. Only one third of them have an HLA identical sibling donor and around 60-70% will find an unrelated donor, thaťs why HAPLO-HSCT offers a therapeutic option to most of these patients with the advantages of quick availability, easy programation and logistics, and a committed donor. Bone marrow (BM) or peripheral blood stem cells (PBSC) could be used as graft source but iťs not established if any of them offer significant advantages.
Materials (or patients) and methods: We retrospectively evaluated the results of HAPLO-HSCT with reduced conditioning regimens and GVHD prophylaxis based on PTCy (50 mg/kg on days +3 and +4) and a calcineurin inhibitor plus mycophenolate from day +5 performed in GETH centers, with focus on the graft source.
Results: From Dec-2007, 118 HAPLO-HSCT have been done in 17 centers. Median age was 36 years (16-67), 64% were males and all were in advanced phases of their disease or presented high risk features (Hodgkiń s 43%, AML/ALL/MDS 36%, NHL/myeloma/others 21%). Previous HSCT had been employed in 64% (autologous 66, allogeneic 19), and in 36% the HAPLO-HSCT was their first transplant. Disease status at HAPLO-HSCT

was CR in 44%, with persistent disease in 55%. BM was the graft source in 48 patients (41%) and PBSC in 70 (59%), non T-cell depleted in all cases. The haploidentical donor was the patient́s mother (28%), father (10%), siblings (44%) or offspring (18%). Baltimorés reduced conditioning (RIC) including 200 cGy was employed in 15% and RIC based on IV busulfan in 85% (44% with 3.2 mg/kg on day -2 (BUX1), and days -3 and -2 (BUX2) in 41%). Median neutrophils engraftment was reached at day +18 (13-45) and platelets >20 K at day +26 (11-150), without significant differences (NS) between BM and PBSC. Main toxic complications were grade II-III muchositis in 36%, febrile neutropenia in 75% and CMV reactivations in 62%. Transplant related mortality rate (TRM) at 1 year was 19% with BM vs 23% with PBSC (NS). Day +100 grade II-IV acute GVHD cumulative incidence (CI) was 46% vs 48%, and grade III-IV was 15% and 10% with BM and PBSC respectively. Chronic GVHD CI at 1 year was 40% vs 24% (NS), being extensive in 16% and 9% (NS) respectively. No differences in acute or chronic GvHD CI were seen when comparing BM against PBSC. After a median follow-up of 10 months (3-61), estimated event-free survival (EFS) and overall survival (OS) at 18 months were 41% and 59% respectively. CI of relapse or progression was 29%. No significant differences in TRM, EFS, OS and relapse incidence were detected between BM and PBSC.

Conclusion: HAPLO-HSCT with PTCy in the treatment of high risk hematologic malignancies, offers long-lasting remissions with manageble toxicity and GVHD, employing either BM or PBSC that render similar results as graft source.

Disclosure of Interest: None declared.

P468

Effect of hypercholesterolaemia on peripheral blood CD34 + cell levels

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Introduction: It was shown that hypercholesterolaemia cause hematopoietic stem cell (HSC) mobilization in mouse models. Different studies that was carried out retrospectively on stem cell mobilization to determine the contribution of cholesterol levels in humans was obtained different results. In addition, these studies were performed in patients diagnosed with hematologic malignancies that can affect cholesterol levels. Therefore, we aimed to determine the peripheral blood CD34 levels and its contribution to the mobilization in newly diagnosed hyperlipidemia patients without any additional disease.

Materials (or patients) and methods: The preliminary results of our prospective study that was supported by Turkish Hematology Association (Project number: 2013/5) was provided. Peripheral blood CD34 levels (BD FACSAria cell sorter, BD Biosiences) of 31 patients (mean age:52.7, M/F:10/21) who was newly diagnosed with hypercholesterolaemia in endocrinology outpatient clinic was compared with age and sex matched 30 healthy persons (mean age:39.1, M/F:13/17) levels. Results are presented as mean ± standard error.

Results: The mean total cholesterol levels of the patients and the control group were 292.3 ± 18.48 vs 154.1 ± 5.12, mean LDL cholesterol levels 201.5 ± 17.56 vs 83.87 ± 4.13, mean HDL levels 53.74 ± 3.14 vs 50.0 ± 2.82 and the mean triglyceride levels were 183.8 ± 17.03 vs 103.3 ± 9.41, respectively. Compared with the control group, peripheral blood CD34 + cell levels were significantly higher in the patient group (1.24 ± 0.13 vs. 1.78 ± 0.19, $P = 0.02$, respectively).

Conclusion: According to the preliminary results of our ongoing study, hypercholesterolemia can contribute to the mobilization of stem cells. In particular, new strategies can be planned for the patients who failed mobilization.

Disclosure of Interest: None declared.

P469

Haploidentical T-Replete Hematopoietic Stem Cell Transplantation in patients with high risk hematological malignancies: A single centre report of 19 cases

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Introduction: Haploidentical hematopoietic stem cell transplantation (Haplo-HSCT) is an alternative treatment for patients with high risk haematological malignancies who lack HLA full-matched donors.

Materials (or patients) and methods: We evaluate the results of 19 patients with high risk hematological malignancies who had recieved a Haplo-HSCT in Hospital Ampang between Feb 2012 and Nov 2014. All grafts were unmanipulated granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells from direct family donors. The first 8 patients received myeloablative conditioning regimen which include 12 Gy of total body irradiation (TBI) in 6 fractions over 3 days and Fludarabine 30 mg/m^2/d for 5 days. They received GVHD prophylaxis which include an antithymocyte globulin(Thymoglobulin) at 2.5 mg/kg/d for 3 days from day-3 to day-1; IV Cyclophosphamide at 50 mg/kg/d on Day + 3 and +4, and IV cyclosporine at 3 mg/kg/day in 2 divided doses on Day +5 onwards. The subsequent 11 patients received myeloablative conditioning regimen which include one fraction of 2 Gy TBI, Fludarabine 30 mg/m2/d for 6 days and IV Busulphan 130 mg/m^2/day for 4 days. The GVHD prophylaxis consisted of an antithymocyte globulin (Thymoglobulin) at 2.5 mg/kg/d for 2 days on Day-3 and Day-2, IV cyclosporine at 3 mg/kg/day in 2 divided doses, started on Day -1 and IV Methotrexate 15 mg/m^2 on Day +1 and 10 mg/m^2 on Day +3, +6 and +11.

Results: Nineteen patients with the median age of 20 years (range from 15y to 43 y) were evaluated in this study. Sixteen patients had underlying acute leukemia, 1 had plasma cell leukemia, 1 had Hodgkin Disease and 1 had Chronic Myeloid Leukemia. Median cell dose of 11.6 x 10^6/kg (range 5.7 to 12.8) CD34+ stem cells. All of these patients attained successful neutrophil and platelet recovery. Median neutrophils and platelets engraftment were 15.5 days and 12.5 days respectively. During the follow-up at median time of 22 months (range 4 to 33 months), 7 (36.8%) patients developed aGVHD grade I-II and 2 (10.5%) patients developed aGVHD grade III-IV. The incidence of cGVHD was 5.2%. Five patients died due to transplant-related causes and four died due to relapse of the underlying disease. There was a higher incidence of regimen-related toxicity in the first 8 patients who received TBI-Flu regimen.

Conclusion: The results suggest that mega dose of unmanipulated G-CSF mobilized peripheral blood stem cells is an appropriate stem cell source for Haplo-SCT and it provides an important strategy for patients who lack HLA full-matched donors.

Disclosure of Interest: None declared.

P470

Retrospective comparison of three mobilization regimens of peripheral blood stem cells in patients with multiple myeloma: efficacy and toxicity

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Introduction: OBJECTIVES OF THE STUDY:

The study purpose is to compare the efficacy and toxicity of three regimens used for mobilization of peripheral blood stem cells (PBSC) in patients with multiple myeloma:Cyclophosphamide, Etoposide and G-CSF alone.

Materials (or patients) and methods: Patients with de novo symptomatic MM aged less than 65 years and eligible for autologous stem cell transplantation (ASCT) were included From January 2003 to October 2014. After induction therapy, they underwent PBSC mobilization with Cyclophosphamide: 4 g/m^2 in day 1 followed by daily administration of G-CSF 5 μg/kg started on day 3 and continued throughout the apheresis period ($CY + GCSF_5$, group1), ETP: 375 mg/m^2/d for 2 days followed by daily administration of G-CSF 10 μg/kg started on day 3 and continuing throughout the apheresis period ($ETP + GCSF_{10}$, group2) or GCSF alone: 10 μg/kg daily until the end of collection ($GCSF_{10}$ alone, group3). The aim is to collect a minimum number of 4.10^{6}CD34 + cells/kg to permit realization of double ASCT. Monitoring of blood cell count and CD34 + circulating cells was started on day 11, 8 and 4 in $CY + GCSF_5$, $ETP + GCSF_{10}$ and $GCSF_{10}$ groups respectively. PBSC collection was performed when the peripheral blood CD34 cell count was > 20/μl and leucocyte count was > 1.10^9/l. Two types of machine were used: Cobe spectra and MCS. Adverse events: neutropenia and thrombocytopenia grade 3-4, fever requiring antibiotics and hemorrhagic cystitis were assessed.
Results: Three hundred fifty eight patients were included. The median age was 53 years (24-65y), sex-ratio was 1,37. Dexamethasone with thalidomide was the induction regimen used in 86% of patients. The median time between diagnosis and mobilization was 6 months (2-29 m). The outcome of cytaphereses and PBSC yields for each group are summarized in Table I.

Table I. Results of Stem Cell Collection

	Total (n = 358)	*$CY + GCSF_5$ (n = 205)*	*$ETP + GCSF_{10}$ (n = 118)*	*$GCSF_{10}$ (n = 35)*	P
Mobilization failure: n (%)	22 (6%)	10 (4,5%)	2 (2%)	10 (29%)	$<10^{-4}$
Peripheral blood CD34 cell count < 20/μl	16 (4,5%)	7 (3%)	1(1%)	8 (23%)	
PBPC < 2.10^6 CD34/Kg	4 (1%)	1 (0,5%)	1 (1%)	2 (6%)	
Other cause of failure	2 (0,5%)	2 (1%)*			
Number of patient having cytapheresis	*n* = 340	*n* = 196	*n* = 117	*n* = 27	
Median number of apheresis	2 (1- 4)	2 (1- 4)	1(1-4)	3 (1-4)	$<10^{-4}$
median time mobilization/apheresis (days)	11 (9-25)	11 (10 - 25)	11(9-17)	day 4 *n* = 25	
Median CD34 + cells yield (10^6/kg)	9,77 (0,42-76,81)	8,89 (1,68 -76,81)	12,98 (0,42-72)	4,19 (0,56-41,27)	$<10^{-4}$
CD 34 cells ≥ 4. 10^6/kg	322 (90%)	185 (90%)	116 (98%)	21 (60%)	$<10^{-4}$

*lost sight, septic shock

$Grade_{3\text{-}4}$ neutropenia was observed in 55%, 34% and 0% of patients in group1, 2 and 3 respectively ($P = 0{,}0001$). $Grade_{3\text{-}4}$ thrombocytopenia was observed in 17,5%, 12% and 3% of patients ($P = 0{,}047$). Thirty two patients required red cell transfusion in group 1(16%), 5 patients in group1 (4%) and no patient in group3 ($P = 0{,}001$). Twenty six patients required platelet transfusion in group1 (13%), 13 in group 2(11%) and one patient (3%) in group3 ($P = 0{,}23$). Antibiotic use for fever in 37%, 8% and 3% of patients respectively in group1,2 and 3 ($P = 0{,}0001$). Hospitalization for fever was needed in 21%, 3% and 3% of patients respectively. Hemorrhagic cystitis occurred only in group 1(2%). No toxic death related to mobilization was observed.
Conclusion: $ETP + GCSF_{10}$ has been superior to $CY + GCSF_5$ and $GCSF_{10}$ alone for achieving collection goals and was less toxic than $CY + GCSF_5$.
Disclosure of Interest: None declared.

P471
Similar Graft-Versus-Leukemia Effect from HLA-haplo-identical and HLA-identical Sibling Donor grafts in Hematopoietic Stem Cell Transplantation

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Introduction: Haplo-identical transplants are increasingly used in hematopoietic stem cell transplantation (HSCT), but it is currently unknown whether they have a stronger graft-versus-leukemia (GVL) effect.
Materials (or patients) and methods: We analyzed 10,679 patients with acute leukemia undergoing HSCT from an HLA-matched sibling donor (MSD, $n = 9{,}815$), or a haplo-identical donor (≥ 2 HLA-antigen disparity, $n = 864$) between 2007–2012, reported to the European Group for Blood and Marrow Transplantation. In a Cox' regression model, acute and chronic graft-versus-host disease (GVHD) were added as time-dependent variables. Patients receiving T-cell replete or T-cell depleted grafts were analyzed separately.
Results: On multivariate analysis, there was no difference in relapse probability between recipients of haplo-identical and MSD grafts. This was seen in both T-cell replete and depleted grafts. Factors of importance for relapse among T-cell replete grafts included remission status at HSCT, Karnofsky score ≤80, acute GVHD ≥grade II, and chronic GVHD ($P < 10^{-5}$). Among patients receiving T-cell depleted grafts, advanced disease ($P < 10^{-5}$) and second, compared to first, remission ($P = 0.01$) were the strongest factors for leukemic relapse. Non-relapse mortality was significantly higher in the haplo group versus MSD transplants among patients receiving T-cell replete or depleted grafts ($P < 10^{-5}$). Leukemia-free survival was superior in the MSD group transplanted with T-cell replete ($P < 10^{-5}$) and T-cell depleted grafts ($P = 0.0006$).
Conclusion: The risk of relapse was the same in acute leukemia patients receiving haplo-identical donor grafts as in those subjected to MSD transplants, suggesting a similar GVL effect.
Disclosure of Interest: None declared.

P472
Are there any optimal cell doses for graft repopulation in the Bone Marrow-Derived Allogeneic Hematopoietic Stem Cell Transplantations?

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Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HCST) is a well-established procedure of many hematological diseases. Fast and sustained engraftment is an

indicator of transplantation success. Accordingly, lower limits on the number of transplanted cells have been defined to ensure safe treatment. The number of total nucleated cells in the graft originated from bone marrow for the transplantation has been widely used successfully as a marker to obtain hematological recovery after the allo-HCST. We aim to present the correlation of nucleated, mononuclear and CD34+ cells with engraftment kinetics in our bone marrow (BM) derived allo-HCST patients.

Materials (or patients) and methods: We present 139 adult patients with benign or malign hematological diseases who underwent the first allo-HSCT from an HLA-matched identical sibling by using bone marrow as stem cell source between September 1998 and January 2014.

Results: Median age of the patients (82 M; 59 F) was 27 years (range: 16-57 ys). Their initial diagnoses at the pretransplantation were 55 chronic myeloproliferative neoplasm, 46 acute leukemia, 32 BM failure and 5 other hematological diseases. 122 of patients received myeloablative whereas 17 had reduced intensity conditioning regimen (RI). After the bone marrow harvest, erythrocyte cell depletion was performed in 21 products mostly due to blood group mismatch. Infused median volume, median nucleated cells (NCs), median mononuclear cells (MNCs) and median CD34+ cells were 1029 mL (45-1728 mL), 2.57x10e8/kg (0.5-8.59), 0.68x10e8 (0.17-6.8) and 2.5x10e6/kg (0.32-8.7). There were positive correlations among NCs, MNCs and CD34+ cells as expected. When the cut-off values were set at 2.5x10e8/kg for infused NC dose, the presence of engraftments for neutrophil and platelet were statistically similar ($P = 0.9$)(Table 1). In addition, similar engraftment days for neutrophil and platelets were observed between low and high NCs. When we excluded the patients who received RI conditioning regimen, there were no differences detected for hematological recovery (Table 1). When the cut-off values for the NCs were separated as $\leq$2.0 vs 2-3 vs $\geq$3.0x10e8/kg, the statistical analyses did not show any differences. When the similar analysis were repeated for low or high CD34+ cells or MNCs as according to the median value, we found no statistical differences.

Table 1 Engraftment kinetics in BM-derived allogeneic stem cell transplantation

All patients	*NCs ($\leq$2.5x10e8/kg)*	*NCs (>2.5x10e8/kg)*	P
The presence of engraftment			
Neutrophil (>0.5x10e9/L)	95.3%	94.1%	0.76
Platelet (>20x10e9/L)	95.0%	92.6%	0.80
Median engraftment days			
Neutrophil (>0.5x10e9/L)	16 (12-32)	16 (5-44)	0.44
Neutrophil (>1.0 x10e9/L)	17.5 (14-44)	17 (6-51)	0.77
Platelet (>20x10e9/L)	22 (0-68)	21 (0-69)	0.75
Platelet (>50x10e9/L)	27.5 (0-148)	28 (0-183)	0.99
Myeloablative group			
The presence of engraftment			
Neutrophil (>0.5x10e9/L)	96.7%	93.2%	0.44
Platelet (>20x10e9/L)	95.0%	91.5%	0.49
Median engraftment days			
Neutrophil (>0.5x10e9/L)	16 (12-32)	16 (11-44)	0.69
Neutrophil (>1.0 x10e9/L)	18 (14-44)	17 (11-51)	0.96
Platelet (>20x10e9/L)	22 (0-68)	22 (0-69)	0.72
Platelet (>50x10e9/L)	28 (0-148)	28 (0-183)	0.98

Conclusion: In conclusion, this retrospective study has not determined any optimal doses of TNCs (more or less 2.5x10e8/kg) which shorten the period of graft repopulation after BM-derived allo-HSCT in contrast to previous studies.

Disclosure of Interest: None declared.

P473
Erythrocyte Depletion of Bone Marrow in Stem Cell transplantation in a Pediatric Center Comparison of two Systems

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Introduction: ABO incompatible stem cell transplantation has an incidence of about 20 - 50%. To avoid side effects from hemolysis bone marrow is erythrocyte depleted. One possible procedure is the erythrocyte depletion with conventional apheresis systems. In our center we use this approach for ABO incompatible bone marrow transplants, but also for volume reduction if the bone marrow amount is inadequately to high (> 30 - 40 ml/kg bw) for unmanipulated transfusion. Recently the OPTIA™ System was introduced with a predefined program for bone marrow volume and erythrocyte depletion. We compared the data from our old system (Cobe spectra™) with the new OPTIA™ System in the framework of our JACIE quality system.

Materials (or patients) and methods: We retrospectively analysed our erythrocyte depletion procedures from 2008 to 2014. In total 32 procedures were performed in 32 patients with various diagnosis undergoing allogeniec stem cell transplantation with HPC-BM as the stem cell source. 21 were performed with the COBE Spectra and 11 with the OPTIA system. The procedures were performed according to the manufacturer's instructions. In 8 cases the bone marrow was prediluted with recipient and donor compatible pRBCs due to the very small volume of the original harvested bone marrow. The mean age of the recipients for both systems was comparable ($n =$ 21, 8,64 +/- 2,58y COBE Spectra; $n = 11$, 6,24 +/- 2,45y OPTIA). Samples were taken pre and post procedure to perform HPC, leukocyte, erythrocyte, platelet, and lymphocyte counts. For all this parameters the collection efficiency was calculated. The charts were reviewed for engraftment data. For comparison of the groups the T-Test was applied.

Results: In both groups the preprocedure counts were comparable and not statistically different. The calculated collection efficiencies were for the most cell counts statistically not different between COBE spectra and OPTIA (HPC 98% versus 103%, $P = 0.57$; Monocytes 96% versus 83%, $P = 0.25$; Granulo 21% versus 34%, $P = 0.1$; B-Lymphocytes 98% versus 94%, $P = 0.67$; T-lymphocytes 104% versus 99%, $P = 0.5$; platelets 49% versus 70%, $P = 0.08$; total nucleated cells 40% versus 50%, $P = 0.14$) but a highly significant difference in the collection efficiency for erythrocytes (5.5% versus 1.4%, $P = 0.001$) resulting in a significant lower erythrocyte volume after the OPTIA procedures (pre Hct 30% versus 29%, $P = 0.58$; post Hct 15% versus 4%, $P < 0.001$; Erythrocyte volume in the product after the procedure 14.5 ml versus 3.7 ml, $P <$ 0.001). The erythrocyte volume pre procedures was not different (291 ml versus 257 ml, $P > 0.05$). 17/21 patients (1 died before, 3 data are missing) in the COBE Spectra group showed regular engraftment and 10/11 in the (1 died before engraftment). The day to reach >1 G/l leukocyte count in the peripheral blood was not statistically different (d +20 versus d +19, $P = 0.56$).

Conclusion: The erythrocyte depletion of bone marrow with OPTIA system is regarding the leukocytes and leukocyte subpopulations as efficient as the procedure with the COBE spectra, even in pediatric patients. The OPTIA system showed lower erythrocyte contamination, which is superior to the older system and also superior to Fenwal CS 3000 plus and AMICUS (V.Witt, JCA, 2011) if we compare our former published data with this data.

References: Witt V. J Clin Apher. 2011;26(4):195-9

Disclosure of Interest: None declared.

P474

Allo-HSCT following RIC regimen for elderly patients (60 years and older) with hematological malignancies using unrelated donors: a retrospective study on behalf of the french society for stem cell transplantation and cell therapies (SFGM-TC)

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Introduction: Reduced intensity conditioning (RIC) regimen allowed extension of allogeneic hematopoietic stem cell transplantation (allo-HSCT) to a previously unserved population of older patients for whom hematologic malignancies are more common. The use of unrelated donors (URD) in patients aged of 60 years or more has drastically increased in the past few years. To date, there are only limited data on the feasibility and outcomes of URD allo-HCT in elderly patients (60 years or more). The purpose of the current study is to describe outcomes in a large cohort of patients aged 60 years or older who received a RIC URD allo-HSCT in recent years.

Materials (or patients) and methods: Between 2008 and 2012, this retrospective multicenter study involved 539 consecutive patients aged of 60 years or more (142 aged 65 or more and 9 aged 70 or more) who received a first allo-HSCT for hematological malignancies (351 with myeloid disorders and 188 with lymphoid malignancies) from an URD after a RIC regimen (Peripheral blood stem cells in 92% of cases) in France. Conditioning regimen was fludarabine-based in 85% of the patients and Graft versus Host Disease (GvHD) prophylaxis consisted of cyclosporine (CSA) plus MMF in 47% of cases and CSA plus methotrexate in 20%. To address the role of age by itself in our population, 2 groups of patients were defined: patients with age at allo-HSCT less than 65 years old ("URD $<$ 65 group", $n = 397$) and patients who were aged of 65 years old or more ("URD $\geq$ 65 group", $n = 142$). This study was approved by the scientific committee of the SFGM-TC and is in accordance with the declaration of Helsinki for clinical research.

Results: Patient characteristics were similar between the 2 groups, for time between diagnosis and allo-HSCT, gender, disease (myeloid versus lymphoid), disease status (complete remission at allo-HSCT versus others), source of stem cells, number of infused total nucleated and CD34 cells, donor age, donor gender, patient/donor sex mismatch, HLA matching, patient/donor CMV status, the use of antithymocyte globulins (ATG) or TBI-based regimen (2 gray only), and GvHD prophylaxis. Patients in the URD $\geq$ 65 group received more CD3 cells ($P = 0.02$). The median follow-up was 36 months (range, 0.3-73.5) for URD $<$ 65 group and 32 months (range, 0.03-72) for URD $\geq$ 65 group. During evolution, the cumulative incidence (CI) of grade II–IV acute GvHD was 36% in URD $<$ 65 group and 31% in URD $\geq$ 65 group ($P = 0.684$) while the CI of chronic GvHD at 2 years was 42% and 35%, respectively ($P = 0.334$). CI of treatment related mortality (TRM), disease free survival (DFS) and overall survival (OS) were not different between the 2 groups (Table 1). Multivariate analysis for TRM, DFS and OS show that age by itself has no influence.

Table 1: 2-year cumulative incidence of TRM, DFS and OS

Characteristics	*TRM*	*DFS*	*OS*
All patients	29%	42%	49%
Patients aged less than 65 years (URD $<$ 65 group)	28%	41%	49%
Patients aged 65 years or more (URD $\geq$ 65 group)	33%	47%	51%
P value	0.350	0.417	0.809

Conclusion: These data suggest equivalence of outcome between URD $<$ 65 group and URD $\geq$ 65 group after RIC URD allo-HCT in this large cohort of elderly patients (60 years or more) with hematological malignancies. Age by itself thus appears not to be a limitation in this particular population of elderly patients.

Disclosure of Interest: None declared.

P475

The combined impact of amino acid polymorphism at HLA-DPB1 on T cell alloreactivity defines a functional distance between patient and donor independently predictive of survival after 10/10 matched unrelated HSCT for AML and MDS

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Introduction: We have previously experimentally determined the differential impact of individual amino acid polymorphisms on the alloreactive T cell response to HLA-DPB1*09:01, translated these findings into numeric "functional distance" (FD) scores for all HLA-DPB1 alleles, and showed that these FD scores correlate with T cell epitope groups determining non-permissive mismatches in unrelated HSCT (*Fleischhauer et al., Lancet Oncol 2012; Crivello et al., Biol Blood Marrow Transplant 2014*).

Materials (or patients) and methods: Here we investigated a new algorithm for non-permissive HLA-DPB1 mismatches in unrelated HSCT, based on numeric DFD scores calculated as the difference between the combined FD scores of the two HLA-DPB1 alleles in patient and donor. A cohort of 268 patients (pts) (age median 56 yrs [18–73 yrs]) who underwent HLA-A,B,C,DRB1,DQB1 matched but DPB1 mismatched unrelated HSCT for AML ($n = 229$ [85%]) or MDS ($n = 39$ [15%]) at the Dept. of Bone Marrow Transplantation of the University Hospital Duisburg-Essen were included in the analysis. FD scores of HLA-DPB1 alleles in patient and donor were calculated as previously described, and the DFD scores for each transplant were calculated as the absolute number of [$FD_{patient}$-FD_{donor}]. Receiver Operator Curves (ROC) were constructed to calculate the best cut-off values for the endpoint overall survival (OS). Statistical association between DFD scores and OS was analyzed using Kaplan-Meier estimates and log-rank tests.

Results: Over the observation period of 8 yrs (median follow-up of 4 yrs for surviving patients), OS in the entire cohort was 47% (95% $>$ confidence interval [CI] 40% – 54%) and ranged from 55% (95% $>$ CI 43% – 65%) for pts in early disease stages ($n = 105$ [40%]) to 42% (95% $>$ CI 33% – 51%) for pts in advanced disease stages ($P < 0.04$). DFD score distribution in the 268 pairs ranged from 0.01 to 7.46[DWB1], with a median of 1.64. ROC analyses indicated stratification into 3 subgroups with DFD scores $<$1.03 ($n = 86$ [32%]), 1.03-2.93 ($n = 118$ [44%]), and $>$2.93($n = 64$ [24%]) as best predictor. In these subgroups, the Kaplan Maier probabilities of OS were 53% (95% $>$ CI 40% – 65%), 47% (95% $>$ CI 36% – 58%) and 38% (95% $>$ CI 25% – 52%), respectively ($P < 0.05$). In multivariate analysis, independent predictors of OS were time-dependent cGvHD ($P < 0.0001$), the use of anti-thymocyte globulin ($P < 0.0001$), aGvHD ($P < 0.002$), patient age ($P < 0.004$) and the DFD score ($P < 0.006$).

Conclusion: In this monocenter study cohort, the HLA-DPB1 DFD scores of patient and donor were a highly significant, independent risk factor for mortality after 10/10 matched unrelated HSCT for AML and MDS, possibly reflecting the strength of the alloimmune response to mismatched HLA-DPB1 in this setting. Association with clinical endpoints non-relapse mortality, GvHD and relapse is under way. If confirmed in larger cohorts, these findings could pave the way for a new approach to the identification of non-permissive HLA mismatches in unrelated HSCT based on an experimentally defined numerical weighting system for amino acid sequence polymorphism in patient and donor.
Disclosure of Interest: None declared.

P476
Unmanipulated haploidentical transplantation with post-infusion cyclophosphamide in poor prognosis haematological malignancies: a survey from 2 centers

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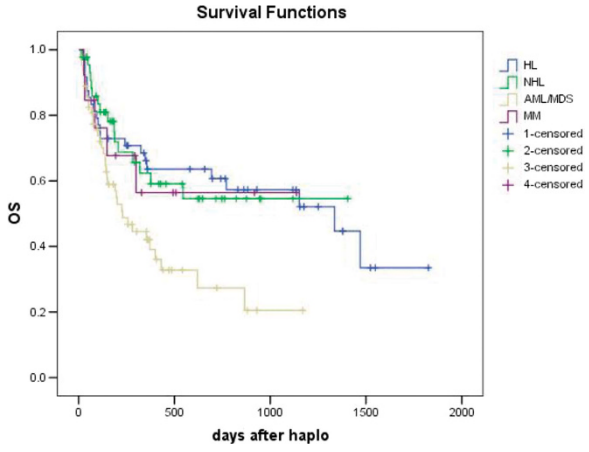
Introduction: Haploidentical transplantation (haplo-SCT) with post-infusion cyclophosphamide (PT-Cy) is becoming a promising treatment for haematological malignancies.We analyzed the outcome of 190 consecutive patients receiving haplo-SCT with PT-Cy at 2 institutions (Institut Paoli Calmettes, Marseille, France and Humanitas Cancer Center, Rozzano, Italy) since 2009.
Materials (or patients) and methods: Conditioning regimens were nonmyeloablative/RIC in 169, and myeloablative in 21 patients. Graft versus host disease prophylaxis (GVHD) consisted of cyclophosphamide (50 mg/kg/day) at d + 3 and + 4, plus cyclosporine A or tacrolimus and mycophenolate mofetil from day + 5. Stem cell source was bone marrow in 90 and peripheral blood stem cell in 100. The median age was 53 years (19-73). Lymphomas were the most frequent disease (n 101), followed by AML/MDS (n 57), and myeloma (n 13). 96 patients were in complete remission before transplantation. 22 patients received a previous allogeneic transplantation. Regarding AML/MDS group, 46% has active disease at time of haplo-SCT.
Results: The median follow-up was 16 months (1-61). The median time to absolute neutrophil count > 500 and platelet count more than 20000 was 20 days (14-49 days) and 29 days (10-395 days), and the engraftment cumulative incidence for ANC at 30 days and platelet count at 60 days was 93% and 91%. Graft failure was diagnosed in 4 patients. The grade 2-4 acute GVHD incidence was 32% and chronic GVHD was 18% (moderate/severe 5%). For all patients, the 2-year overall survival and progression free survival were 51% (43-59%) and 47% (43-51%). The 2-year NRM and Relapse rate were 22% (16-28%) and 30% (23-37%) respectively. OS by diseases is showed in the figure.



Conclusion: This retrospective study on haplo-SCT with PT-Cy suggests that 1) NRM is similar to allogeneic transplantation from HLA identical donor and lower than other alternative donors; 2) the speed of engraftment is acceptable 3) The acute and chronic GVHD incidence was low and notably severe forms were rare. In particular, advanced chronic forms were rare; 4) haplo-SCT is effective, even if patients' population was heterogeneous precluding firm conclusions; 5) worse outcomes in myeloid malignancies may probably related to disease stage and need further developments.
Disclosure of Interest: None declared.

P477
Mobilization of hematopoietic progenitor stem cells in allogeneic setting with lenograstim by subcutaneous injection, in daily or twice-daily dosing: a single-center prospective study with historical control

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Introduction: Allogeneic hematopoietic progenitor stem cells transplantation (HPSCs) is an established procedure for many haematological disease. Although the mobilization of peripheral blood HPSCs from Healthy Donors (HDs) using G-CSF is widely used, the ideal method for the administration of the citokine has not been determined.
Materials (or patients) and methods: 75 consecutive related HDs received lenograstim (LENO) as G-CSF mobilization agent. LENO was given s.c. at a dose of 10 mg/kg in a once-daily dose (ODD) every 24 hours. Results were compared with a historical control group of 181 HDs treated with 5 mg/kg LENO twice-daily dose (TDD) with a time interval of 12 hours.
Results: HDs characteristics were similar in both the ODD and TDD treatment groups. The peripheral blood WBC count on day 4 and on day 5 after G-CSF administration reached 39.6 ± 14.1 and 40.4 ± 13.8 x10E9/L, respectively, in the ODD group *versus* 40.6 ± 11.4 and 43.4 ± 11.7x10E9/L in the TDD group (P = ns). The peripheral blood CD34 + cell concentration evaluated on day 4 and on day 5 was 45/μL (range, 6 -217) and 75/μL (range, 7 - 279) with ODD, respectively versus 36/μL (range, 3-200) and 55(range, 3–738) with TDD ($P = 0.067$, and $P = 0.001$). The collected CD34 cell count in first apheresis was 5.6 ± 2.9 106/kg body weight donor in the ODD versus 5.4 ± 3.8 in the TDD cohort, respectively ($P = 0.08$). 5 HDs (6.7%) mobilized CD34 + cells < 2 x 10E6/kg recipient body weight in the ODD group compared with 7 HDs (3.9%) in the TDD group ($P = 0.3$). Bone pain was the most common short-term adverse event that was reported by 86.6% of the donors in ODD group versus 71.2% in the TDD group ($P = 0.005$). No severe grade 4 toxicity was observed.
Conclusion: once-daily administration of LENO is at least effective as twice-daily administration for the mobilization of CD34 + cells in HDs. Peripheral blood CD34 + cell count on days 4 and 5 suggest that the once-daily LENO administration schedule should be more effective than split dose for the mobilization in HDs
Disclosure of Interest: None declared.

P478

Mobilization of peripheral blood stem cells with lenograstim in healthy related donors >50 years old: efficacy and safety analysis

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Introduction: Over the past 15 years, the landscape of allogeneic hematopoietic stem cell transplantation (HSCT) changed from being rarely performed in patients 50 years or older to accounting for a little less than half of the transplantations reported. An old patient usually has an old sibling healthy donor (HD). The safety and efficacy of G-CSF for mobilization in elderly HDs has not been provided. In our institution a long-term active follow-up study of G-CSF-mobilized HDs has been implemented since 1998

Materials (or patients) and methods: After a median follow up of 94 months (7.8 years) we reviewed and analyzed safety and efficacy data on our HD database according to HD age: HDs-1 (162), patients younger than 50 years old, HDs-2 (62), patients aged 50-59 and HDs-3 (23), patients aged 60 or over. Long-term follow-up included the monitoring of neoplastic, cardiac or autoimmune diseases. Primary efficacy endpoint was the peak count (number) of CD34+ cell at day 4 and 5 after mobilization with G-CSF (lenograstim)

Results: Two hundred and forty-seven successive HDs were evaluated and their characteristics are well balanced among age-groups and no statistical differences were detected: most of them were male (55.9%), sibling (97.2%) and HLA matching (93.1%). Short- and long-term safety was not different among age-groups. Bone pain was reported as the most frequent short-term adverse event (76.5%). For long-term safety surveillance, the observed rate of solid tumors, haematological malignancies, cardiovascular and autoimmune events was the expected incidence for these diseases in the western countries. The median number of CD34+ cells was 44.5/34.5/26 ($P=0.0062$) and 65.5/58/46 ($P=0.4455$), according to age-group, at day 4 and day 5 respectively.

Conclusion: LENO demonstrated to be safe and effective both in the mobilization of young and old HDs after a median FUP of around 8 years. This data contributes to the growing body of evidence of the long-term safety of G-CSF for allogenic donor stem cell mobilization also for elderly HDs.

Disclosure of Interest: None declared.

P479

Which unrelated donor should I choose?

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Introduction: Sometimes there are two or more unrelated donors to choose between when trying to find the best unrelated donor for a patient. It is still not clear which factors are most important in selecting an unrelated donor.

Materials (or patients) and methods: All consecutive patients transplanted between 1995 and 2014 for a malignancy with a HLA-A, -B and –DRB1 matched unrelated donor that were additional typed for HLA-C, -DPA1 and –DQB1 and treated with HSCT at Karolinska University Hospital were included. Most patients (55%) had acute leukemia while 16% had MDS and 16% chronic leukemia. The median age was 44 years (<1-72). All patients received ATG as part of the conditioning.

Results: Totally 504 patients were included. A 12/12 match was used in 199 patients, 11/12 in 223, 10/12 in 71 and 8-9/12 match was used in 11 patients. We found no significant effect of the match on overall survival. The 5-year OS were 57%, 58%, 52% and 45% and RFS were 49%, 55%, 50% and 40% in the four groups, respectively. However, patients with an 8-9/12 matched donor tended to have inferior OS and RFS compared to the other groups ($P=0.12$). Among the 11/12 matched donors, 70 had a HLA-C MM, 123 a DPA1 MM and 30 a DQB1 MM. No effect on OS (62%, 57% and 54%) and RFS (57%, 55% and 52%) was found depending on type of MM in these 11/12 matched pairs. Among the 10/12 matched pairs, we found inferior OS and RFS when a HLA-C+DPA1 MM ($n=37$) occurred compared to all other combinations ($n=34$), 37% vs. 68% and 35% vs. 68%, $P=0.02$. In multivariate analysis of factors related to the donor, we found that donor age (per decade)(RH 1.45, $P<0.001$), CD34+ dose (per 10^6/kg)(RH 1.05, $P=0.003$) correlated to inferior OS while the use of PBSC (RH 0.62, $P=0.03$) correlated to superior OS. A CD34+ cell dose $>11x10^6$/kg or $<3x10^6$/kg were associated to lower OS. Grade of match (RH 0.96, $P=0.58$), TNC dose (RH 0.79, $P=0.09$) did not affect OS in this material. For RFS the results were very similar.

Conclusion: When selecting a HLA-A, -B and –DRB1 matched donor a 10/12 match seems to result in similar outcome as 11/12 or 12/12 matches. Multiple mismatches (3-4) should be avoided. The most important factors seem to be donor age and CD34+ cell dose. Younger donors are better and a very high ($>11x10^6$/kg) or very low ($<3x10^6$/kg) CD34+ cell dose should bee avoided.

Disclosure of Interest: None declared.

P480

HLA-A allele mismatch (7/8 or 9/10) is the second best option after 8/8 or 10/10 matched unrelated donors: An analysis on results from Turkish centers

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Introduction: Hematopoietic stem cell transplantation (HSCT) from an unrelated donor has been established as an effective treatment option for patients with hematological diseases who lack a human leukocyte antigen (HLA)-matched related donor. However, HLA mismatch at the genetic level (allele mismatch) may be observed among serologically HLA-matched (antigen match) donor-recipient pairs, which adversely affects the incidence of severe graft-versus-host disease (GVHD) and survival. The aim of this retrospective multicenter study was to evaluate the impact of HLA mismatch on unrelated transplantation outcomes in Turkey.

Materials (or patients) and methods: The data set consisted of follow-up records of 444 (of which 436 with HLA matching data available) unrelated-donor stem cell transplantations performed at 14 centers between July 2002-September 2014 and facilitated by TRAN orTRIS.215 patients underwent single antigen and/or allele-mismatched (mm) HSCT. The distribution of the mismatches according to the HLA-A, HLA-B, HLA-C, and HLA-DR and HLA-DQ loci are:82, 58, 32, 35 and 9 patients, respectively.Twelve patients were transplanted with 8/10 HLA matching. The patients' characteristics are summarized in Table 1.

Results: The neutrophil engraftment was achieved in 82.2% of the patients. HLA mm has a negative impact on engraftment (HLA mm: median 17days vs HLA-matched: median 16 days,

$P = 0.03$). Acute GVHD wasobserved at a rate of 42.1%. HLA matching did not have an impact on the incidence of acute GvHD ($P = 0.35$) but chronic GvHD was more frequent among HLA allele /antigen mismatched patients than HLA-matched ($P = 0.008$). The possibility of 5-year overall survival (OS) was 50.2% ± 2.5%. The presence of HLA mismatch significantly shortened the OS (58.9 ± 3.4% vs Allele mm: 49.8 ± 5.7% vs Antigen mm 36.0 ± 4.9%, $P < 0.0001$).Among the allele level mm HLA-A mm was associated with better OS compared to other loci (55.9 ± 11.7%vs. 17.2 ± 8.2%). When analysis was performed regardless of HLA match or only among HLA matched donor-recipient-pairsgender and stem cell source (PB vs BM) did not have an impact on OS. The OS of patients transplanted between 2002-2007 were shorter than those transplanted later (2008-2014)(37.9 ± 6.4% vs. 52.9 ± 2.7; $P = 0.02$).

Table 1 The Patient characteristics

	HLA-identical	*1Antigen mismatch*	*1 Allele-mismatch*
Median age (years)	21 (1-62)	24 (3-65)	30 (2-62)
Recipient gender (F/M)	83/138	50/78	34/53
Donor gender (F/M)	66/152	58/70	33/54
Diagnosis			
Acute leukemia	87	67	39
Lymphoma	12	9	8
BM failure	51	11	12
CMPN	18	0	9
MDS/MPN	13	9	9
Immune	21	0	3
Deficiency	17	11	6
Inherited	1	1	1
disorders	7	0	0
Others			
Missing data			

Conclusion: Matching for HLA was possible approximately in half (211/436) of the unrelated transplants performed since 2000.Mismatches were associated with later engraftment, more chronic GVHD and shorter OS. HLA-A provided the most acceptable mismatch at allelic level.Donor gender was not found to be as powerful as HLA. Experience of transplant centers have improved the survival outcomes through the last decade.
Disclosure of Interest: None declared.

P481

Prospective donor outcome follow-up: results and challenges of the first 6 years of Swiss experience

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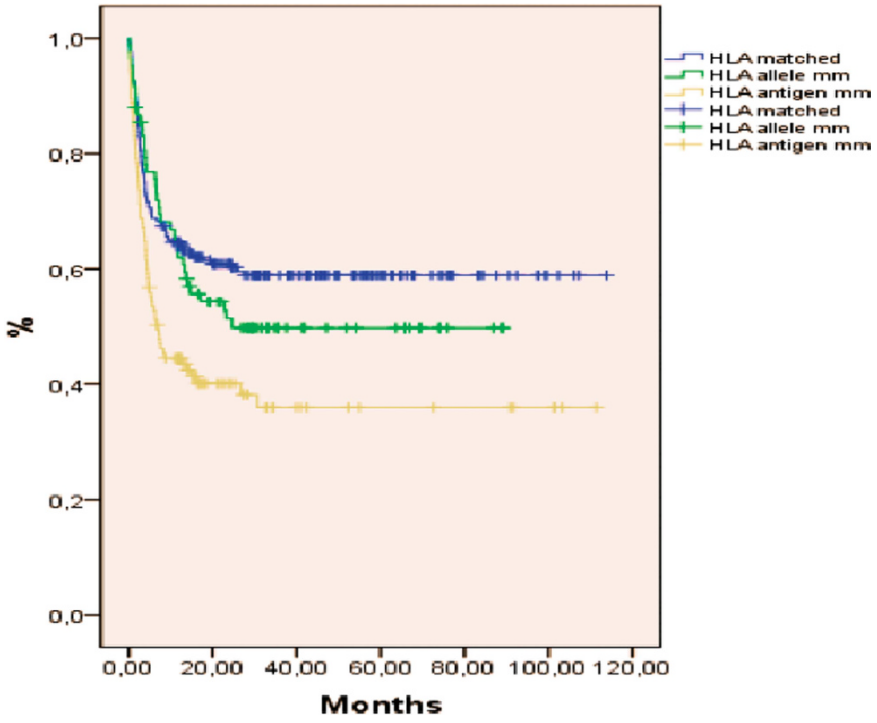
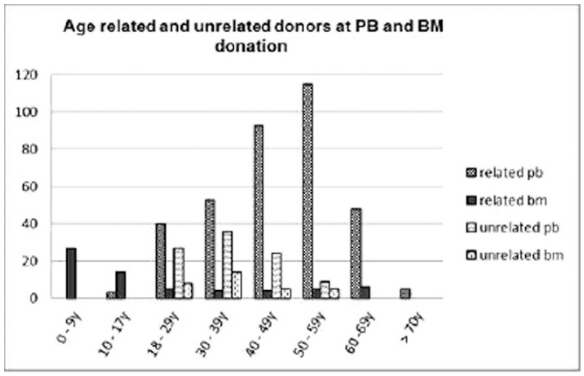
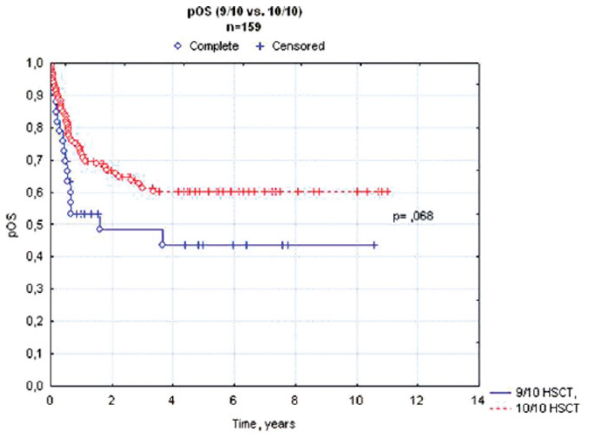
Introduction: Donor follow-up (FU) data of unrelated donors (URD) have been collected since 1988 in Switzerland (CH) by the Swiss blood stem cell donor registry, Swiss Blood Stem Cells (SBSC). Standardized FU of related donors (RD) began in 2007, when FU was harmonized for RD and URD.
Materials (or patients) and methods: Since July 1st 2007 data on every haematologic stem cell (HSC) donation performed by URD and RD in CH are collected prospectively in the EBMT database ProMISe. Data include donor details, type of collection, number of donations, pre-existing health disorders, complications during and after collection and FU data. Until August 30th 2013 the time-points of data collection were: time of harvest, 1 month (mo), 6mo, 1-5-10 years (y) post donation, then every 10 y.
Results: From July 1st 2007 - June 30th 2013 a total of 578 HSC donations took place in the 4 Swiss collection centres (CC): 448 by RD and 130 by URD. Of these, 552 were first (424 RD/128 URD), 25 second (23 RD/2 URD) and 1 a third donation (RD). The further analysis includes only the first donations: 424 were done by RD (66 bone marrow (BM), 358 peripheral blood stem cells (PB) and 128 by URD (32 BM, 96 PB). 227 (54%) of the RD and 51 (40%) of the URD were female (f) donors. Median age of RD was 8y higher than of URD (47,7y (range 6mo–74y) vs 39.6y (18-55y)). Furthermore the age distribution showed marked differences (fig).

[P480]







Pre-existing health disorders were more frequent in RD 145/424 (34%) vs URD 12/128 (9%). The most frequent were cardiovascular (RD 51/145 (35%) vs URD 3/12 (25%), haematological (14 non-malignant, 1 low grade NHL detected immediately post-donation; RD 15/145 (10%) vs URD 0) and psychological disorders (RD 14/145 (10%) vs URD 0). No donation-related severe adverse events (SAE) were reported.
During FU later than 6mo the following events were reported: 1 autoimmune disease 4 years post-donation in 1 URD; in RD: 1 breast malignancy, 1 melanoma and 1 bronchial asthma all at 1 year post-donation, 1 basal cell carcinoma and 1 case of MGUS 5 years after donation.
Of the 424 first donations by RD, 351 had 1mo FU and 379 donors had at least one FU during the first year resulting in an overall FU rate of 89% in the first year with large differences between CCs. From 128 URD first donations, 124 had a 1mo FU. 100% of URD were seen at least once in the first year. Since 2007 the Swiss national society and SBSC unified the donor FU procedure. For want of resources in the CCs, FU management was transferred to SBSC in April 2012. Since then, the general FU coordination is managed by SBSC, 1mo FU is performed by the CCs, later FUs by SBSC. The optimization of resources and administration allowed to improve the FU rate in all Swiss CCs, as illustrated most obviously in the CC with the previously lowest return rate: from 25% to 100% at 1mo FU, from 44% to 77% at 6mo FU and from 50% to 73% at 1y FU.
Conclusion: Donor characteristics differ greatly between RD and URD concerning age and pre-existing health disorders, illustrating the need for RD FU, since FU data from URD are not representative for RD. Donor FU is very good at 1mo for both RD and URD. From 6mo ongoing, the FU is more challenging for many CCs but can be improved by implementing collaboration strategies with partners that can provide expertise in organization and FU. In our case this knowhow is provided by SBSC. This kind of collaboration may be a model for other countries too.
Disclosure of Interest: None declared.

P482
10/10 match unrelated donor hematopoietic stem cell transplantations can improve outcomes in comparison with 9/10: single-center study

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Introduction: MUD HSCT is a routine method now. We retrospectively evaluated the influence of HLA match (9/10 vs 10/10) on the major transplant outcomes.
Materials (or patients) and methods: 159 HSCT from 9/10 and 10/10 performed during the period 2003-2014. Diagnosis: AML – 43% (68), ALL – 19% (31), AA – 12% (24), inherited disorders of metabolism – 10% (16), PID – 4% (6), other – 8% (14). Gender distribution: male – 67% (107), female – 33% (52). Age median – 7,6 y.o. (1-17 y.o.). Stem cell source: BM – 77% (122), PBSC – 23% (37). 126 pts. (79%) received 10/10 HSCT and 33 pts. (21%) – 9/10 HSCT. Conditioning regimen contained different agents according to protocol and type of disease.
Results: Overall incidence of a.GvHD (I-IV st.) in 10/10 group was 83% (107) and in 9/10–82% (27), a. GvHD III-IV st. incidence – 17,5% (22) and 21% (7) accordingly. We revealed that the type of GvHD prophylaxis could significantly improve the outcome: Tacro/MTX prevention was better in comparison with other types (Tacro/MMF and others). Tacro/MTX scheme decreased severe grade 3-4 a.GvHD episodes in both groups: pts. with 10/10 – from 16% to 1,5%; 9/10 group – from 18% to 3%. Relapse/rejection rate for 10/10 was 12% ($n = 15$) and 18% ($n = 6$) for 9/10 (no statistical difference). Infection episodes were not significantly different in both groups. In 10/10 group at median follow up of 11,2 years, the estimated probability of OS was 60,2% and in 9/10 group OS was 41,8% (median follow-up – 10,8 years).
Conclusion: Our results suggest that 10/10 transplants have better outcome and lower incidence of severe a. GvHD. But our experience showed that 9/10 transplant results improved in past several years. Now 9/10 transplants is a good option in case of absence of 10/10 donor.
Disclosure of Interest: None declared.

P483
Post-thaw viable CD45 + cell dose and clonogenic potential of CD34 + are associated with better engraftment and outcome after single umbilical cord blood transplantation in adult patients with malignant disease

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Introduction: Allogeneic stem cell transplantation (Allo-SCT) using single cord blood unit (CBU) is a well established strategy when a patient lacks HLA compatible related or unrelated donor. While the quantity of cells is widely accepted as the main factor influencing the outcome of this kind of Allo-SCT, the impact of the quality of the CBU has been less studied. We analyzed the quality characteristics of grafts and studied their possible correlation with the transplant outcomes.

Materials (or patients) and methods: We included all consecutive patients who received an Allo-SCT with a single CBU between January 2005 and December 2013 at 3 different institutions, using the same stem cell laboratory to process the cells. Variables analyzed were: post-thaw total cell numbers (TNC, CD34+,CD3+ and CFU) and those corrected by viability [viable CD45+, viable CD34+, viable CD3+ cells dose and expected clonogenic efficiency (eCLONE, ratio of post-thaw CFU between pre-freeze CD34+)].
Results: A total of 110 adult patients underwent a single umbilical CB transplantation during the study period (median age 35 years, range 18-55). All patients received myeloablative conditioning based on thiotepa (10 mg/kg), busulfan (6.4 mg/kg i.v), fludarabine (150 mg/m^2) and anti-thymocyte globulin (6 mg/kg). The diagnoses were: AML ($n=56$), ALL ($n=26$), lymphoma [non-Hodking's and Hodking's ($n=17$)], CML ($n=6$) and others ($n=5$). Median follow-up for survivors was 49 months (13-114). Median cell dose after thawing for TNC and total CD34+ was 2.6 x 10^7/kg (1-5.3) and 1.1 x 10^5/kg (.6-4.8) respectively. Median cell dose of viable cells after thawing for CD45+, CD34+ and CD3+ was 2 x 10^7/kg (0.5-4.8), 1 x 10^5/kg (2-4.2) and 4.9 x 10^6/kg (1-5.3) respectively. Median dose of CFU and resulting eCLONE was 3.7 x 10^4/kg (0.1-16) and 22% (.6-64). At a median time of 60 days, the cumulative incidence (CI) of engraftment was 83% (95% CI:76-90) for neutrophils and 76% (95% CI:68-86) for platelets. Primary graft failure was observed in 11 patients. At 5 years, the CI of non-relapse mortality and relapse was 39% (95% CI:29-49) and 19% (95% CI:12-18) respectively. Main causes of death were opportunistic infections ($n=23$) and primary graft failure ($n=8$). The probability of disease-free survival (DFS) and overall survival (OS) at 5 years were 40% (95% CI: 31-51%) and 41% (95% CI: 32-52%). In multivariate analysis a number$\geq$2 x 10^7/kg of post-thaw viable CD45+ cell dose was significantly associated with faster myeloid ($P=.01$) and platelet engraftment ($P=.01$), better DFS ($P=.01$) and OS ($P=0.02$). In addition, an eCLONE$\geq$20% was associated with better results for myeloid ($P=.005$) and platelet engraftment ($P=.01$) and non-relapse mortality ($P=P=.02$).
Conclusion: Our study demonstrates that viable CD45+ and eCLONE may add significant information to the classical CBU selection criteria based on absolute cell counts and are associated with outcomes after CB transplantation.
Disclosure of Interest: None declared.

P484
X-MAP Luminex technology is an effective strategy for allele-level HLA typing of CB units. The experience of the Pavia CBB

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Introduction: For improving the quality of banked Cord Blood (CB) units a winning strategy could be to perform HLA typing at medium/high definition level at time of banking. Recent literature (*Eapen M, 2014*) has suggested the importance of high-level donor–recipient match at class I and class II HLA loci for the outcome of unrelated CB transplantation (CBT). In particular, allele-level matching at HLA-A, -B, -C, and -DRB1 was found to be associated with the lowest non relapse mortality after CBT in patients affected with acute leukemia and myelodysplastic syndrome. Several well-known molecular techniques, such as PCR-SSP and PCR-SSO, are available as useful tools to type CB DNAs. In this setting Luminex platform (LABScan 100 xPonent, One Lambda, Canoga Park, California) may represent a new approach able to perform HLA analysis in a quick, accurate and cost-effective manner.
Materials (or patients) and methods: Starting from January 2014, we have been typing both CB units and mothers by Xmap Luminex technology, which consists of a reverse PCR-SSO ($n=88$, CB units typed for HLA-A, -B, -C, and -DRB1 and $n=88$, mothers typed for HLA-A, -B and DRB1).
Results: By retrospective analysis, we found that the benefits of SSO Xmap Luminex are clear and consistent in terms of reduction of testing time and quality improvement of results. In particular, the time between testing and listing CB is shorter in the first six months of 2014 compared to 2013 (155 days vs 185 days respectively). In fact, traditional approaches consisted of a two-step typing (low resolution SSO followed by high resolution SSP), while Luminex technique directly provides an allelic result. Moreover, this platform allows to simultaneously process a large number of DNA locus-specific samples (more than 90) with a reduced quantity of DNA (2 ml at 20 ng/ml are needed to amplify a genomic locus-specific sequence) in a single working session.
Conclusion: Basic procedural and planning advantages and simplicity of the protocol combined with the operators compliance and satisfaction makes Luminex an effective and easy to apply technology. The impact of high allele-level donor-recipient match at HLA-A, B, C and DRB1 loci on survival after CBT for hematologic malignancies has modified the strategy of donor choice and suggest Luminex technology application in CBB programs. In this setting, we believe that the adoption of such a high performance and simple tool in the Immunogenetics laboratory can really play a role. Defining allele polymorphisms for the whole class I and II loci at time of listing will improve the attractiveness of CBB inventory by reducing the time for donor search and procurement and simplifying donor choice.
References: Eapen M, Klein JP, Ruggeri A, Spellman S, Lee SJ, Anasetti C, Arcese W, Barker JN, Baxter-Lowe LA, Brown M, Fernandez-Vina MA, Freeman J, He W, Iori AP, Horowitz MM, Locatelli F, Marino S, Maiers M, Michel G, Sanz GF, Gluckman E, Rocha V; Center for International Blood and Marrow Transplant Research, Netcord, Eurocord, and the European Group for Blood and Marrow Transplantation. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. Blood. 2014 Jan 2;123(1):133-40.
Disclosure of Interest: None declared.

P485
Knowledge and attitudes of medical students towards hematopoietic stem cell transplant and willingness to donate: A Survey based study

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Introduction: Hematopoietic Stem cell transplant (HSCT) is a curative treatment for many cancers, genetic, and autoimmune diseases and its indications are rapidly expanding. The majority of the HSCTs currently performed utilize an unrelated donor who donates stem cells voluntarily. Although the medical community may be aware of the benefits of being a donor for this life saving procedure, it is unknown what the current attitudes of physicians, nurses, medical students and allied health professionals are regarding the stem cell donation process. A literature gap is identified on the knowledge and attitudes of medical students towards stem cell transplantation, transplant registries and donation, and how this impacts their willingness to, not only join the bone marrow registry, but donate to unrelated patients in need.
Materials (or patients) and methods: Medical students are a young, diverse, influential population whose willingness to engage in altruistic acts such as donating stem cells may be correlated with knowledge on the topic. We performed a cross-sectional survey among 4 year cohort of

medical students at Mayo Medical School in Rochester, Minnesota, USA. The questionnaire evaluated current knowledge of the bone marrow registry, motivation to donate stem cells, and assessed altruistic behaviors via a validated scale, the Altruistic Personality Scale (Fetzer Institute; used with permission).
Results: Responses were received from 99 medical students for a total response rate of 40%.

Age	*SD ± 23.8*	*Race*	*SD ± 22.3*
21-25	54 (56%)	American Indian or Alaskan Native	0 (0%)
26-30	33 (34%)	Asian	19 (20%)
31-35	6 (6%)	South Asian	-5
>36	4 (4%)	Southeast Asian	-2
Gender	SD ± 8.5	Eastern Asian	-7
Male	42 (43%)	Western Asian	-1
Female	54 (56%)	Not specified	-4
Not designated	1 (1%)	Black/African American	4 (4%)
Level of medical education	SD ± 19.4	White	62 (65%)
Pre-Clinical Years 1-2	44 (45%)	Hispanic	9 (9%)
Clinical Years 3-4	37 (38%)	White	-8
MD/PhD	13 (13%)	Black	0
Other	3 (3%)	Other	-1
		Native Hawaiian/Pacific Islander	1 (1%)
			1 (1%)

42% of respondents are currently on the BMT registry. There was an appreciable gender gap between males and females on the registry (29% of males, 55% of females) compared to males and females who had donated blood products (55% of males, 51% of females). Motivation to donate was high among medical students surveyed. However, significant knowledge gaps were identified in donation methods and differences between blood donation and stem cell donation.
Conclusion: This is the first study that we are aware of that evaluates the knowledge and attitudes of medical students about HSCT utilizing a validated tool. Surprisingly, we have identified many gaps in the basic knowledge of the donation process in a substantial number of students. We can consider designing strategies and propose medical school curriculum changes to improve knowledge gaps concerning HSCT in medical students. Ongoing analyses of medical students at other institutions as well as students pursuing other health professions will further elucidate these correlations. A multi-school study evaluating attitudes and knowledge is needed.
Disclosure of Interest: None declared.

P486
Challenges of transplanting patients in an isolated centre: the experience from the South University Hospital of La Réunion Island

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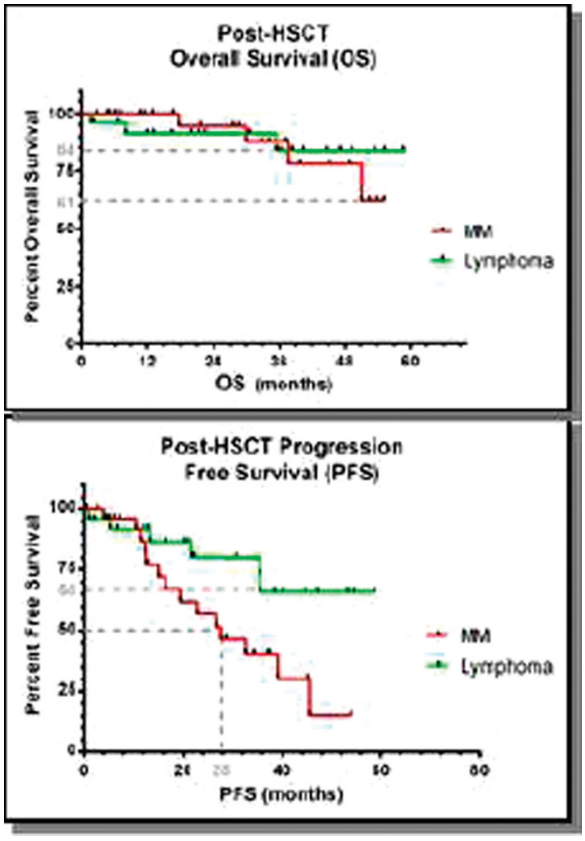
Introduction: Reunion Island is a French territory in the Indian Ocean, with over 934 000 young and multi ethnic inhabitants who all benefit from the same health insurance as in France. Thus, the patients who cannot be treated locally are transferred to France.
Our transplant activity concerns only autografts (autoSCT)for the moment. Hematopoietic Stem Cell (HSC) collections and autoSCT procedures are made in our center, with two senior nurses responsible for transplant coordination. Patients who need allograft (alloSCT) are thereby transferred to France.
The island specific situation generates several difficulties in the management of patients going on alloSCT: geographic distance, separation from family, accommodation in France usually for more than 6 months, climate change, financial difficulties and also lack of donors because of the broad and mixed ethnic backgrounds.
Materials (or patients) and methods: To determine the peculiarities of our situation, we conduct a retrospective mono centric study. We examine here all patients who underwent or needed auto and alloSCT from January 2010 to December 2014 in our center.
Results: In this period, 47 autoHSC collections and 54 autoSCT were performed. Patients treated for Multiple Myeloma (MM) were 52% ($n = 28$) and received a High dose Melphalan conditioning whereas 46% ($n = 25$) received a BEAM conditioning for Hodgkin or non-Hodgkin lymphomas. The autoSCT mortality is null in the MM subgroup and only 1 patient in the Lymphoma group died of central venous catheter septicemia. Concerning grade > 1 adverse events, one patient in the MM group had venous thrombosis and a corticoid-related diabetes occurred in one patient with Lymphoma. The median Progression-free survival (PFS) is 28 months in the MM group and is not reached in the lymphoma group with 68% of patients free from disease at 60 months. The estimated Overall Survival (OS) at 60 months is 61% in the MM group and 84% in the Lymphoma group (median not reached in both groups).
During the same time, 24 patients were eligible for allograft. Mean age was 38 (18-61), with 47% male and 53% female. Diseases were: 61% acute leukemia, 10% chronic leukemia, 5% lymphoma. Over these patients 16 (66%) were actually transferred for alloSCT in France and 8 (33%) did not. The reasons for alloSCT non-performance were: geographical and family isolation ($n = 5$), disease progression ($n = 2$) and lack of donors ($n = 1$). Actually, 7 patients are being followed in our center after alloSCT and 1 is in relapse.
Conclusion: Our retrospective analysis confirms the low rate of transplant-related morbidity and mortality of autoSCT in our daily practice. The median PFS and estimated OS in MM and lymphoma groups in our center are similar to those found in the literature. Concerning alloSCT, a non-negligible number of patients do not benefit from treatment because of the specificities of the population or the isolation.



To reduce this number, we aim to enhance the register of bone marrow donors which actually represents less than 1% of the population and collect cord blood in the existing bank.
Our current challenge is to develop alloSCT and to confirm autoSCT following the standards of care provided by the Joint Accreditation Committee ISCT-EBMT for a better management of these patients, especially taking into account the isolation of the population and the need of tailored and individualized treatment plans.
Disclosure of Interest: None declared.

P487
Minimising Donor Attrition at Work-Up: the BBMR Experience

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Introduction: The time taken to get a patient to a stem cell transplant influences the outcome of that procedure. Unrelated donor registries aim to minimise attrition of donors along the pathway to donation of stem cells. Loss of donors after their selection as the optimal or "final" donor is particularly problematic as there is little time available to find another donor and almost inevitably the transplant is delayed.
Materials (or patients) and methods: We reviewed factors affecting the ability of finally selected donors from the British Bone Marrow Registry (BBMR) to proceed to stem cell donation for patients in international transplant centres (TCs) between January and November 2014.
Results: Excluding donors cancelled for recipient reasons, 130 BBMR donors were selected as final donors. There were concerns about eligibility in 46 (35%) of these donors – "unconfirmed" donors. 13 of these 46 (28%) donors were deferred for medical reasons. In 9 of these, the reasons could not have been predicted ahead of their medical assessment (abnormalities of liver function (2), coagulation (2), ECG (1), neutropenia (3), thrombocytopenia (1)). Three donors failed because of previously undiagnosed hypertension and one, because of investigations for abdominal pain that had onset after medical questionnaire at the time of confirmatory typing. 33 of the 46 (72%) "unconfirmed" donors needed extra interventions and/or communication with the TC. Of these, 24 donors needed extra interventions to confirm their suitability to donate. 9 donors needed extra tests performed (split bilirubin levels 4, iron studies 3, serum protein electropheresis 1, EBV PCR 1) and 10 needed repeat tests (blood count 5, coagulation 2, urinalysis 2, urea and electrolytes 1). 6 needed review of their ECGs by a cardiologist and 1 needed extra radiological investigation. 2 donors visited their family doctors for blood pressure monitoring to prove "white-coat" hypertension. TCs were asked to confirm acceptability of 14 donors: donors with single organ autoimmune disease (4 cases), positive/equivocal EBV Ig M results (3), toxoplasma Ig G positivity (2), allergy history (1), and alpha thalassaemia (1). TCs were involved in risk assessments for 4 donors (possible exposure to infectious agents within 4 months of donation – 1 recent travel to malarial area, 1 tattoo, 1 endoscopy, 1 injected bodybuilding agent). After these interventions, all 33 of these "borderline" donors (72%) proceeded to donate with no or only very minor delay to the transplant schedule.
Conclusion: Extra interventions in unrelated donors are labour-intensive but worthwhile in minimising donor attrition at this late stage. 72% of "unconfirmed" donors could be salvaged by registry medical staff with further follow-up. Communication with TCs is vital. Blood pressure recording before donor medical assessment may further reduce donor loss.
Disclosure of Interest: None declared.

P488
T-replete haploidentical allogeneic transplantation provides promising disease control with low incidence of graft versus host disease in advanced acute myeloid leukemia and myelodysplastic syndromes

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Introduction: T-replete haploidentical donor allogeneic transplantation (HaploSCT) using post transplantation cyclophosphamide (PTCy) allowed achieving very low incidences of severe GVHD, making non-relapse mortality (NRM) comparable to standard donor allogeneic transplantation (AlloSCT). In contrast, it was reported that HaploSCT was associated with a higher incidence of relapse, with few reports specifically focused on myeloid malignancies. However, it is not clear if this statement is due to the procedure itself or to the high risk of the diseases, usually included in the previously published reports. Therefore, we investigated the outcome of patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) undergoing PTCy HaploSCT.
Materials (or patients) and methods: This bi-center study included consecutive patients who underwent HaploSCT for AML/MDS using PTCy from 2011 to 2014. According to institutional guidelines, patients without standard donor were indicated for HapoSCT in case of high risk diseases defined by refractory diseases, high risk cytogenetic diseases or relapse after first AlloSCT. All types of conditioning regimens and graft sources were allowed.
Results: Fifty four patients with AML ($n = 40$) or MDS ($n = 14$) and a median age of 58 years (22-72) were analyzed. Hematopoietic cell transplantation comorbidity index (HCT-CI) was $> = 3$ in 32 patients (59%). Non-myeloablative regimen with fludarabine (Flu), Cy and 2 Gy TBI was given in 18 patients (33%), while the most frequently used myeloablative regimen was based on thiotepa, busulfan and Flu ($n = 19$, 35%).
Ten patients received HaploSCT as second transplant because of relapse ($n = 9$) or graft failure ($n = 1$) after first AlloSCT. Twenty-nine patients (54%) were transplanted with active disease. According to the disease risk index (DRI), patients were classified as low ($n = 3$, 6%), intermediate ($n = 19$, 37%) and high/very high ($n = 28 + 2$, 58%) risks.
Grade 2-4 and 3-4 acute GVHD incidence was 19% and 2% (day 100), respectively. All grades and severe chronic GVHD (1 year) incidence was 15% and 4%, respectively. With a median follow up of 12 months (3-38), 1-year NRM, CIR, PFS and OS were 31%, 31%, 38% and 46%, respectively. The DRI was the major determinant of outcome, with a 1-year CIR of 14% and 40% in the intermediate and the high/very high groups, respectively ($P = 0.02$). This leads to better 1-year PFS (64% vs 28%, $P = 0.006$) and OS (71% vs 34%, $P = 0.01$) in the intermediate risk group. Multivariate model including age, conditioning regimen and HCT-CI confirmed the negative impact of high/very high DRI (CIR: HR, 95CI = 5.4, 1.2-25, $P = 0.03$; PFS: HR, 95CI = 3.1, 1.2-8.1, $P = 0.02$; OS: HR, 95CI = 3.1, 1.2-8.2, $P = 0.02$). We found no impact of age, conditioning regimen or HCT-CI on NRM, CIR, PFS and OS.
Conclusion: These results are promising showing that even in very advanced disease, high disease control can be achieved; in less advanced situation disease control is eventually much better conducting to satisfactory outcomes. Both results indicate that HaploSCT procedure do really exert an efficient tumor control contradicting initial concerns on this matter. We suggest that HaploSCT could be safely extended to patients who lack a standard donor without impairing disease control. We also confirmed the low incidence of severe GVHD after HaploSCT.
Disclosure of Interest: None declared.

P489

Natural Killer (NK) Cell And Natural Killer-T (NKT) Cell Infusion Immediately Following Posttransplant Cyclophosphamide (PTCY) To Improve The Outcome Of Haploidentical HSCT Following Nonmyeloablative Conditioning

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Introduction: Relapse of advanced malignancies remained a major concern following PTCY based Reduced Intensity Haploidentical HSCT. We hypothesized that the antitumour activity of NK cells and the anti-GVHD properties of NKT cells could be utilized to compensate for absence of myeloablative conditioning in these patients.
Materials (or patients) and methods: In a pilot study we carried out Haploidentical PBSCT employing this protocol in 5 patients with advanced hematological malignancies who were not eligible for a myeloablative conditioning. The conditioning consisted of Fludarabine 30 mg/m^2 for 5 days followed by 2 Gy TBI. Patients with myeloid leukemia ($n = 3$) received Treosulfan 10 mg/m^2 and those with lymphoid malignancies ($n = 2$) received Cyclophosphamide 30 mg/kg in two doses. PTCY was administered between 64-72 hours after PBSC infusion. This was followed by cyclosporine administered to maintain a trough level of 100-200 ng/ml. Mycophenolate and Sirolimus use were prohibited in this protocol. NK cells and NKT cells were selected from unstimulated lymphocyte collection using Clinimacs single step CD56 enrichment without CD3 depletion and infused on Day + 7.
Results: The median CD34 + cell dose was 8 x 10^6/kg (range 5-12), CD3 dose 10 x 10^8/kg (range 7-36). The NK cell infusion consisted of CD56 + CD3- cells (median 12 x 10^6/kg, range 5.4-18.8), CD56 + CD3 + cells (median 4.47 x 10^6/kg, range 1.3-9.2), CD56-CD3 + cells (median 0.5 x 10^6/kg, range 0.13-1.7). All patients engrafted with ANC > 500 at a median of 14 days (range 10-17) and platelet > 50,000 at 12 days (range 9-15). Donor Chimerism remained above 95% at all time points. Cyclosporine was tapered and stopped by Day 90 in all. None of the patients had GVHD grade2-4. Only one patient had transient CMV reactivation and none reactivated EBV or Adenovirus. No invasive fungal infection was documented. NK cell count was > 200 cells/mm^3 at day 28 with ligand mismatched KIR phenotype ie KIR 2DL1 for C2 mismatched donor, KIR2DL2/3 for C1 mismatched donor and KIR3DL1 for Bw4 mismatched donors reflecting the percentage detected in the donor. The median CD4 + cell and CD8 + cell counts at days 28, 60 and 90 were 208 cells/mm^3, 244 cells/mm^3 and 247 cells/mm^3 & 260 cell/mm^3, 429 cells/mm^3 and 565 cells/mm^3 respectively. The TREG levels remained low throughout the study period (median 0.57%, range 0.40-0.76). All patients achieved and sustained complete remission until the last follow-up. Two patients with refractory lymphoma were detected to be PET-negative at Day 60 and remained in CR. All patients with myeloid malignancies achieved a CR with complete molecular response.
Conclusion: NK cell infusions have been employed by several groups at various time points before or after transplant with variable results. Our study highlights the feasibility of combined NK and NKT cell infusion immediately after PTCY with prompt engraftment and immune reconstitution without any GVHD and excellent anti-cancer effect. The omission of MMF and Sirolimus with minimal use of G-CSF, all of which prevent NK cell proliferation along with the timing of NK/NKT cell infusion might be responsible for the above findings.
Disclosure of Interest: None declared.

P490

The evaluation of the efficacy and safety of original filgrastim (Neupogen®), biosimilar filgrastim (Leucostim®) and Lenograstim (Granocyte®) in CD34 + peripheral hematopoietic stem cell mobilisation procedures for alloHSCT donors

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Introduction: In this study, we aimed to compare the potency of different G-CSF agents including original filgrastim (Neupogen®), biosimilar filgrastim (Leucostim®) and Lenograstim (Granocyte®) on CD34$^+$ cell mobilisation in patients who underwent allogeneic hematopoieitic stem cell transplantation (alloHSCT).
Materials (or patients) and methods: The data of 243 donors for alloHSCT recipients were analysed, retrospectively. Patients who received Filgrastim (Neupogen®, Group I), biosimilar Filgrastim (Leucostim®, Group II) and Lenograstim (Granocyte®, Group III) were analysed for total CD34$^+$ cell count at the end of mobilisation procedures.
Results: A total of 243 donors and patients for alloHSCT were analyzed retrospectively. The median doses of G-CSF agents (μg/kg/day) in PBSC collection in Neupogen® group was;11.00 (10.0012.00) in Leucostim® group10.35(min-max: 10.00-11.10) and in Granocyte® group11.00(min-max: 10.00-11.00). There was no statistical significance among groups ($P = 0.215$). The median number of total collected PB CD34$^+$ cells (x10^6/kg) was 7.12 (min-max:5.38-7.90) in the Neupogen® group, 7.27 (min-max:6.79-7.55) in the Leucostim® group and 7.15 (min-max:5.34-7.58) in the Granocyte® group. There was no statistically significant difference among groups in term of total collected PB CD34$^+$ cells ($P = 0.919$).
Conclusion: Biosimilar filgrastim (Leucostim®) was found comparable to original Filgrastim (Neupogen®) and Lenograstim (Granocyte®) for PBSC mobilization in donors of the patients that underwent alloHSCT.
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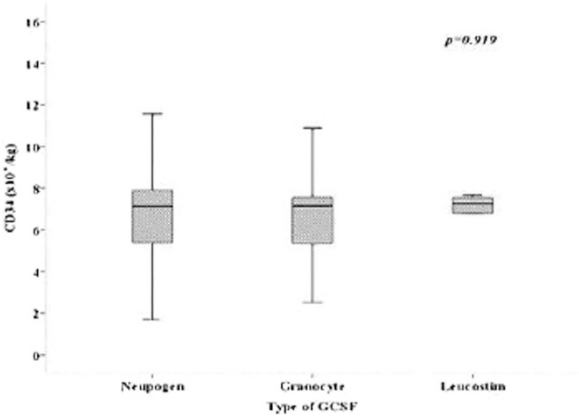
Disclosure of Interest: None declared.



P491

Benefit of KIR alloreactivity based on receptor-ligand model on the clinical outcome of allogeneic hematopoietic stem cell transplantation

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Introduction: Receptors on natural killer (NK) cells, named killer immunoglobulin like receptors (KIRs) recognize HLA class I alleles. Inhibitory KIRs that interact with HLA-Bw4, -C1,and -C2 have known to be most clinically relevant, and failure to recognize the corresponding ligand on a KIR mismatched cell triggers NK cell cytotoxicity.
Materials (or patients) and methods: Patients who received allogeneic hematopoietic stem cell transplantation (HSCT) from either related or unrelated donor in Samsung Medical Center (Seoul, South Korea) between April 2011 and October 2013 were analyzed. KIR mismatch was defined by incompatibility between the donor KIR and recipient KIR ligand (receptor-ligand model), and all cases were divided into 2 broad haplotypes of KIR-A and –B.
Results: he median age was 41 years, patients with acute leukemia ($n = 51$, 86.4%) and myelodysplastic syndrome ($n = 8$, 13.6%) were included. Transplantation from sibling and unrelated donor comprised 28.8% ($n = 17$) and 71.2% ($n = 42$), and peripheral blood was used as a source of stem cells in all patients. Kaplan-Meir survival curves for overall survival (OS), disease free survival (DFS), and cumulative incidence of relapse (CIR) favored recipients with KIR mismatched donor although statistically insignificant. However, in multivariate analysis, KIR mismatch was independently prognostic for better OS ($P = 0.010$, HR = 0.148), DFS ($P = 0.022$, HR = 0.237), and CIR ($P = 0.031$, HR = 0.117). There was no significant difference in OS, DFS and CIR between KIR-A and –B haplotype.
Conclusion: Recipients with KIR mismatched donor showed significantly improved OS, DFS, and CIR. Contrary to expectation, we could not find protective effect of donor KIR B-haplotype.
Disclosure of Interest: None declared.

P492

Characterization of donor peripheral blood stem cells in terms of progenitors, immune cells and impact on engraftment: Analysis of 109 HLA identical (ID) sibling donors

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Introduction: BACKGROUND: The curative potential of allogeneic hematopoietic stem cell transplantation (Allo SCT) is primarily related to immune reaction from the donor to the host. Post-transplant immune reconstitution is donor age related (immune senescence) and determines engraftment, tolerance, graft versus leukaemia effects and graft versus host disease. However, little is known in terms of correlation between immune cells of the graft and host engraftment post AlloSCT.
AIMS: This study describes features of the graft-composition according to donor age, and establishes its relationship with engraftment after Allo SCT.
Materials (or patients) and methods: We retrospectively reviewed 109 evaluable patients (pts) who underwent HLA ID Sibling AlloSCT from Jan 2000 to Dec 2011 in one single Institution.
The immunological content of donor graft was further investigated by Flow cytometry. CD3/CD8/CD4/CD19/CD25/CD56/CD45RA/CD45RO antibodies were used to quantify the absolute counts of T, B and NK cells subpopulations. CD34/CD33/CD38/HLA-DR were used to quantify subpopulations of hematopoietic stem cells. Haematological and Day 90 immunological recovery ($CD4 > 200/mm^3$) was correlated to the amount of infused cells. Statistical analysis was performed by SPSS v20.
Results: RESULTS/DONORS: The median donor age was 42.5 (11-66) years (y).The Sex ratio M/F was1.53. Seventy-nine donors (72.5%) were CMV positive well balanced in all categories of ages. Enough CD34+ stem cell were collected within one day after G-CSF mobilization in one hundred donors (92%). The median of total harvested CD34+ stem cells was significantly decreasing with age. (9.37*10^6/kg in pts < 45y vs 7.3*10^6/kg in pts > 45y ($P = 0.022$)).Among the CD34+ subpopulations, the median number of CD34+33- was weakly decreasing with age. ($P = 0.05$)
The T, B and NK lymphocyte counts as characterized by CD19+, CD4+, CD8+, CD25+, CD45RA, CD45RO and CD56+ did not seem to be influenced by donor age. Female donors produced less CFU-GM colonies than males (160*10^5/c vs 180*10^6/c) ($P = 0.039$).
RESULTS/HOSTS: The median number of infused CD34+ cells was 5*10^6/kg (2.2- 9). The median time to neutrophils engraftment (TNE) was 15d (0-33), and to platelet engraftment (TPE) was 12d (0-108). TNE and TPE don't seem to be correlated to donor age. Negative correlation has been found between the amount of infused CD25+ cells and TNE ($P = 0.033$); and between infused CD34+38- cells and TPE ($P = 0.012$).Positive correlation has been found between CD3+ and TNE ($P = 0.035$).Time to immune recovery after AlloSCT was positively correlated to the amount of CD45 RA+ in the infused graft.($P = 0.05$).The higher amount of NK CD56+ cells was found in the group with shorter TNE (<15d) (2.75*10^6/kg vs 1.14*10^6/kg) ($P = 0.023$), but no correlation has been statistically confirmed.
Conclusion: +Our observations confirm that donor age was significantly associated with a lower number of CD34+ and CD34+33- progenitor cells and female donors seem to produce less CFU-GM.
+Our results suggest that the optimal sibling graft in term of engraftment and immune recovery would come from a young male donor with a higher amount of CD34+,CD25+ and CD33+38- cells but a lower content of CD3+ andCD45RA+cells.
Disclosure of Interest: None declared.

P493

Efficient mobilisation of peripheral blood progenitor cells from adult sibling donors using a short course of G-CSF

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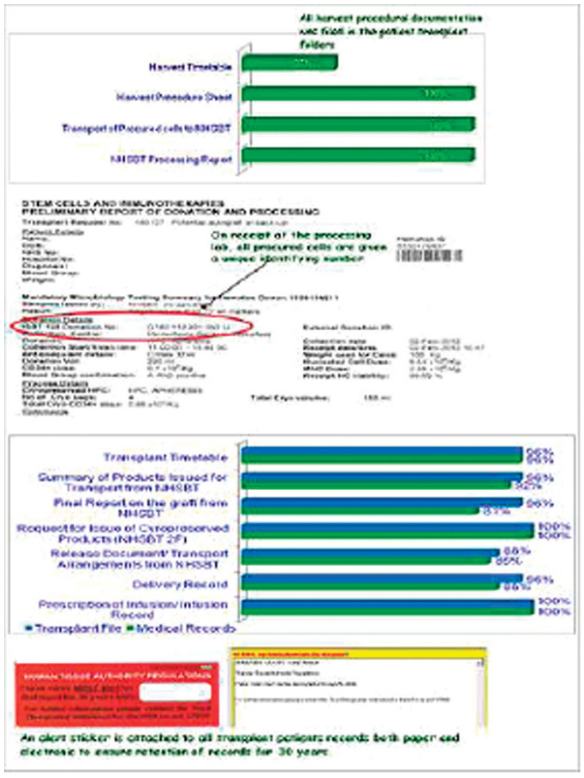
Introduction: We evaluated the feasibility of a short G-CSF mobilisation of peripheral blood progenitor cells from adult sibling donors, used consecutively at our centre since 2000.
Materials (or patients) and methods: The donors were scheduled to receive G-CSF 5 ug/kg s.c. twice daily for three days. Peripheral blood WBC and CD34 counts were measured on the morning of day 4, *i.e.* immediately before the 7th G-CSF dose. If the CD34 count was above 10/μl, apheresis (volume 12-17 l) was started. The goal was to harvest $\geqslant 4 \times 10^6$ CD34+ cells per kg recipient weight, with a minimum acceptable yield of 2×10^6/kg for grafting.
Results: 95 sibling donors (52 males and 43 females) at a median age of 47 (range 20-71) years were treated according to this scheme, receiving a median G-CSF dose of 10 (range 7-12.5) ug/kg/day. On the morning of day 4 the median WBC $\times 10^9$/l was 41 (range 19-77) and the median CD34/μl was 50.8 (range 7-203), with 93/95 donors exceeding a CD34 count of 10/μl. Apheresis was initiated on day 4 in 91/95 donors. Two donors with a CD34/μl of <10 on day 4 were harvested on day

5 and 6 with a total CD34 yield of 2.8 and 3.4 x10^6/kg, respectively. Two donors with a CD34/μl of >20/ul on day 4 were harvested on day 5 for logistical reasons, with a CD34 yield of 5.6 and 9.3 x10^6/kg, respectively. In 91 donors starting apheresis at day 4, the median CD34 yield of a single harvest was 5.3 (range 0.6-15) x10^6/kg. The yield was ⩾2 x10^6/kg in 86/91 donors, and ⩾4 x10^6/kg was harvested in 65/91 donors. In 24/26 donors harvested with <4 x10^6/kg on day 4 (median CD34 yield 2.7, range 0.6-3.8 x10^6/kg), a second apheresis following continued G-CSF administration was performed on day 5 (median CD34 yield 2.8, range 0.6-6.7 x10^6/kg). Notably, only 5 of these 24 donors had a >50% better yield on day 5 than on day 4. However, in 23/24 donors a total yield of ⩾4 x10^6/kg after two days of collection was reached, and no one underwent a third apheresis. One donor failed completely to mobilise (1.2 x10^6/kg in two days apheresis) and underwent bone marrow harvest. One additional donor received plerixafor following the day 4 harvest (yield 1.4 x10^6 CD34/kg) due to contraindication to bone marrow collection, and was harvested on day 5 with a CD34 yield of 4.6 x10^6/kg.

Conclusion: Mobilisation with G-CSF 5 ug/kg twice daily allowed apheresis at day 4 with sufficient yield of peripheral blood stem cells after only one collection day in the majority (>70%) of adult sibling donors. Compared to the "standard" mobilisation regimen (G-CSF 10 ug/kg once daily for 5 days), the mobilisation period was reduced by one day and the use of G-CSF was reduced by 30% for these donors. The majority of "poor mobilisers" were not significantly better mobilised on day 5 than on day 4, but in all but one donor the target yield was reached after no more than two collection days.

Disclosure of Interest: None declared.



P494

AUdit of Traceability in Autologous Stem Cell Transplantation

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Introduction: Traceability is ensured by unique identifiers at key stages of the transplantation process that are interconnected in specific documents. This ensures complete traceability of stem cells from donor to recipient even if they are one of the same. A unique identifying code must be allocated to the donor and the donated tissues and cells, during procurement or at the tissue establishment. To ensure proper identification of the donor and the traceability of all donated material, a register of codes must be kept.

Materials (or patients) and methods: Aims & Objectives: To ensure documentation is evident and filed in the patient transplantation file and /or medical records of the traceability of cells from donor to recipient. **Sample:** Patients who had peripheral blood stem cells procured and transplanted at Mid Yorkshire 2012/2013.

Results: There were 31 harvests and 26 transplants undertaken in the patient cohort. All harvest procedural documentation was filed in the patient transplant folders. On receipt at the processing lab, the procured cells are given a unique identifying number. Transplant documentation available in either patient records and/or transplant folder.

Conclusion: All harvest documentation was found in either the transplant folder and/or medical records. There were a small number of transplant documentation missing from the patients records including 4% (1/26) final report on graft, summary of products issued for transplant, transplant timetable and stem cell delivery record respectively. In 11% (3/26) of patients the release/transport documentation was not found in the patients records. However all these patients had a record of the delivery of cells on the ward. Patient's procured cells are given a unique identification number by the NHSBT in-line with processing procedures. This unique number is checked at every stage of the transplantation process. All records are kept for a minimum of 30 years in line with the HTA standards.

References: ·JACIE 5th Standard – C7.3.1/D7.3.1 ·HTA (Human Tissue Authority) Act 2004 ·Human Tissue (Quality and Safety for Human Application) Regulations 2007.

Disclosure of Interest: None declared.

P495

Feasibility of PBSC harvest on day 4 with the Spectra Optia in healthy stem cell donors

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Introduction: Peripheral blood stem cells (PBSCs) used for transplantation are collected by apheresis after pre-treatment of healthy donors with G-CSF. Reports indicate that the Spectra Optia® Apheresis System (Terumo, Germany) harvests stem cells more efficiently than the previously used COBE® Spectra, which led to the hypothesis that it may be possible to limit G-CSF administration to donors (4 instead of 5 days) and perform the collection a day earlier (Flommersfeld *et al.*, abstract EBMT 2014). Based on a theoretical exercise, we aimed to assess feasibility of day 4 collection in our center and whether donor factors predictive for success could be identified.

Materials (or patients) and methods: All donors (related and unrelated) received 10 μg/kg Filgrastim once a day for 3 days consecutively and on day 4 twice daily. On the 5th day the collection took place. CD34 measurements were performed on day 4 and day 5 in all donors. We analysed 43 stem cell apheresis procedures on the Spectra Optia between June 2013 and June 2014 to measure the collection efficiency (CE). CE was calculated according the formula: CE2 = CD34 in product /(pre procedure CD34 * processed blood volume). The median CE and maximum processed blood volume (16200 ml) were used to predict the amount of CD34x10^6/kg in the product when the apheresis procedure had taken place on the 4th day of G-CSF

Donor counts and CD34 harvest on day 4 (predicted) and 5 (actual)		
	Day 4	Day 5
	median (range)	median (range)
Donor counts (N = 106)		
• WBC (x10^9/L)	34.0 (18.6 - 63.3)	48.3 (25.7 - 87.5)
• CD34 (x10^3/ml)	29.2 (5.8 - 139)	65.6 (18.7 - 212.6)
• CD34 % WBC	0.08 (0.02 - 0.34)	0.14 (0.045 - 0.44)
• CD34 % MNC	0.57 (0.12 - 3.04)	0.94 (0.11 - 4.75)
CE2 (%)		Optia: 53.1 (27.9 – 77.4) Cobe 49.5 (23.4 – 75.2)
Yield CD34 (x10^6/kg)	3.0 (0.72 - 58.9) total	Optia: 6.2 (2.3 - 30.6) Cobe: 6.4 (1.6 – 21.6)
Success (%)	21.7	Optia: 69.8 Cobe: 60.3

administration. To enlarge the sample size, we added CD34 day 4 counts from a historical cohort of 63 donors (apheresis on the Cobe Spectra). The predicted values were compared to the requested amount of CD34/kg (median 5 (4 -20 x 10^6 CD34/kg) in order to assess success. Subsequently, we analysed which parameters would predict sufficient mobilization.

Results: Using the median CE from 43 recent procedures (53.1%), the predicted success on day 4 would have been 21.7% compared to 60.3% on day 5 for the Cobe procedures and 69.8% for the Optia procedures (see table). The group from which we would have harvested sufficient cells at day 4 ($n = 23$) had a higher concentration of CD34 cells: 57 (17.1-139) x10^3/ml and more thrombocytes 228 (187-318) compared to donors ($n = 83$) in which harvest would be insufficient (CD34 cells 23 (6-110) x 10^3/ml and thrombocytes 198 (119-377)). The groups did not differ in gender, BMI, weight and age.

Conclusion: PBSC harvest on day 4 instead of 5 would be feasible in 21.7% of donors but is not predictable. Unlike a previous report, G-CSF reduction seems therefore not feasible in our collection center.

Disclosure of Interest: None declared.

P496

Comparing treatment outcome of allogeneic stem cell transplantation according to the stem cell source: How to manage if donor from Korean Marrow Donor Program is unavailable?

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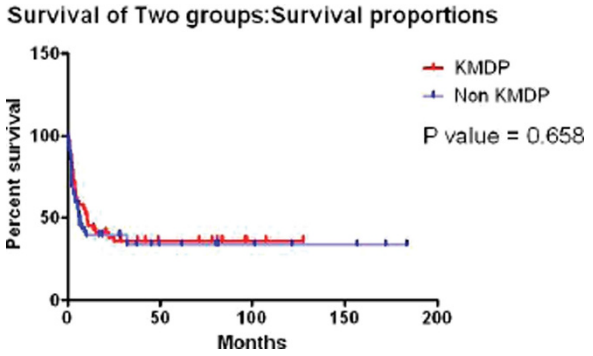
Introduction: Results of allogeneic stem cell transplantation (ASCT) from unrelated donors are considerable when donorsand patients are matched in HLA locus for more than 80%. Due to the special situation in Korea, where Koreans consistof homogeneous population, investigation of result of ASCT from foreign country donors are necessary. In this study, wetried to analyze outcome of ASCT according to the donor nationality.

Materials (or patients) and methods: We retrospectively reviewed medical records of patients who received unrelatedASCT in Seoul National University Hospital from 2005-2014. We performed matched case-control study to adequatelycompare the outcome of ASCT from foreign country donors with that from Korean donors (Korean Marrow DonorProgram, KMDP). The matching variables are age, gender, diagnosis, disease status at the time of ASCT, andconditioning regimen. Retrospective analysis of clinical outcome including overall survival (OS) was performed usingmedical record review.

Results: A total of 43 patients underwent transplantation using stem cells from foreign country donors (Cohort A). Thedetail of donor source are; Australia ($N = 1$), Taiwan ($N = 4$), China ($N = 3$), Japan ($N = 28$), America ($N = 9$), and Germany($N = 1$). Disease included Acute Biphenotypic Leukemia ($N = 2$), Acute Lymphoblastic Leukemia ($N = 8$), Acute MyeloidLeukemia ($N = 12$), Chronic Myeloid Leukemia ($N = 5$),



Myelodysplatic syndrome ($N = 5$), Myelofibrosis ($N = 1$), ParoxysmalNocturnal Hemoglobinuria ($N = 1$), Severe Aplastic Anemia ($N = 8$), and T cell prolymphocytic leukemia ($N = 1$). Matchedcohort who received ASCT from KMDP donors was generated from a pool of 43 patients (Cohort B). When survivalanalysis was performed, median overall survival (OS) from the date of transplantation was months 6.46 in Cohort A, and10.56 months in Cohort B. Cumulative OS rate at 5 years was 0.338 in Cohort A, and 0.359 in Cohort B ($P = 0.6581$). When we compared 5-yr OS rate according to the underlying disease, little difference was observed in acute leukemiapatients with median OS of 21.7 months in both groups. In contrast, patients who received ASCT for MDS showsignificant difference, 50% in cohort A versus 16.7% in cohort B. In SAA patients, 5-yr OS rate was 75% in cohort Aversus 62.5% in cohort B. When race of 10 donors from western countries (Germany, America and Australia) werestudied, 5 donors were Asian and 5 donors were non-Asian. In OS analysis of these 10 patients, no significant differencewas notified according to donor race (31.8% 5-yr OS rate for Asian race donors, and 40% 5-yr OS rate for non-Asiandonors, $P = 0.866$). In cohort B, 15 patients received ASCT before 2004 and 7 of them are surviving (OS rate of 0.467). Incontrast, only 7 patients are surviving among 29 patients who received ASCT after 2004 (OS rate of 0.241). This may beattributable to increased stem cell donation in Korea, making a bias according to the transplantation year.

Conclusion: No statistically different OS outcome was observed between Cohort A and Cohort B. And survival plot shows tendency for improved OS in Cohort B. So. multi-center study is necessary to confirm this phenomenon.

References: High-resolution HLA matching in hematopoietic stem celltransplantation: a retrospective collaborative analysis, Daniel Fürst et al., Blood, 2013, 122; 3220-3229

Disclosure of Interest: None declared.

P497

Multi-center feasibility analysis of unmanipulated haploidentical transplantation in patients with myelodysplastic syndrome: a viable alternative but disadvantageous to identical sibling transplantation

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Introduction: Encouraging results from small sample of haploidentical haploidentical hematopoietic stem cell transplantation (HSCT) for patients with myelodysplastic syndrome

(MDS) need to be confirmed and the risk factors identified in HLA-identical transplant need to be explored in haploidentical setting. Algorithm deriving from outcome comparison between haploidentcial and those of contemporaneous alloHSCT using HLA-identical sibling donors (ISDs) need to be established

Materials (or patients) and methods: We analyzed outcomes of 142 consecutive MDS patients undergoing unmanipulated haploidentical HSCT at 6 centers excluding those with transformation to acute myeloid leukemia. Outcomes were compared with results in 96 patients who received transplants from ISDs during the same period. Multivariate analysis using a Cox model were undertaken to identify risk factors for transplant outcomes. This study was registered as NCT01793675 at www.Clinicaltrial.gov

Results: For patients undergoing haploidentical and ISD transplantation, 5-year cumulative incidences of non-relapse mortality were 32% and 21% ($P = .030$) and of relapse were 4% and 6%, respectively (P not significant [NS]). 5-year probabilities of survival were 63% and 74% ($P = .031$) and of LFS were 62% and 72% ($P = .049$), respectively. And a multivariate analysis show a marked trend of significant differences in NRM and survival rates between the 2 cohorts (both $P = .059$). LFS correlated significantly with patient age of more than 50 years old ($P = .019$), disease duration before transplant ($P = .040$), using bone marrow alone as graft source ($P = .032$) and grades III to IV acute GVHD ($P < .001$).

Conclusion: Unmanipulated haploidentical HSCT was confirmed to be a valid alternative for patients who lack an identical donor for patients with MDS and younger patients with earlier disease course obtain better survival. There is no advantage to using a haploidentical versus an identical sibling donor.

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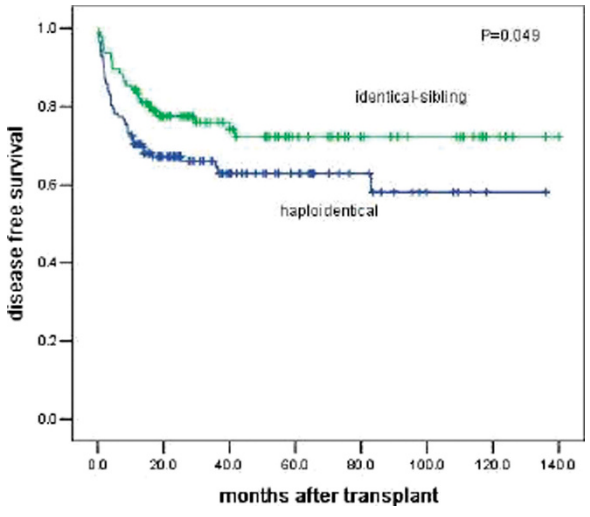
Disclosure of Interest: None declared.



Graft-versus-host disease – preclinical and animal models

P498

Blocking TWEAK-Fn14 interaction inhibits hematopoietic cell transplantation-induced intestinal cell death and reduces GvHD

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Introduction: TNFα serves as a crucial mediator in intestinal cell death and graft-versus-host-disease (GvHD). TWEAK, the TNF-related weak inducer of apoptosis, sensitizes for TNFα-induced cell death via its receptor fibroblast growth factor-inducible 14 (Fn14, TNFRSF12a). Therefore, we addressed the therapeutic potential of blocking TWEAK-Fn14 interactions to prevent intestinal GvHD after allogeneic hematopoietic cell transplantation (allo-HCT).

Materials (or patients) and methods: We employed a recombinant Fn14-specific blocking human IgG1 antibody variant with compromised antibody-dependent cellular cytotoxicity (ADCC) activity in myeloablative conditioning based MHC major mismatch allo-HCT mouse models of acute GVHD. Effects of blocking Fn-14 were further evaluated in graft-versus-leukemia (GvL) mouse models of A20 B-cell lymphoma and genetically induced IM-380 plasmablastic lymphoma.

Results: First, we observed Fn14 upregulation in histological samples from patients with intestinal GvHD. Next, utilizing an Fn14-specific blocking antibody strongly inhibited the severity of murine GvHD. Treatment of the allo-HCT recipients with this mAb reduced cell death of gastrointestinal cells. Yet, blocking Fn-14 neither affected organ infiltration by donor T-cells nor cytokine production. Furthermore, Fn14 blockade also inhibited intestinal cell death in mice challenged with TNFα. This suggests that Fn-14 blockade predominantly exerted its effect via protection of intestinal cells from TNFα-induced apoptosis rather than via immune suppression. Importantly, Fn14 blockade did not impair graft-versus-leukemia (GvL) activity.

Conclusion: Blocking Fn14 with ADCC-defective antibodies may also be useful for the treatment of various inflammatory conditions where TNFα-induced cell death is relevant. Based on our preclinical data we propose blocking Fn14 with ADCC-defective antibodies as a potential novel GvL effect-sparing therapy for the treatment/prevention of GvHD.

Disclosure of Interest: A. Beilhack: None declared, M. Chopra: None declared, A. Brandl: None declared, D. Siegmund: None declared, A. Mottok: None declared, V. Schäfer: None declared, M. Biehl: None declared, S. Kraus: None declared, C. Baeuerlein: None declared, M. Ritz: None declared, K. Mattenheimer: None declared, S. Schwinn: None declared, A. Seher: None declared, T. Grabinger: None declared, H. Einsele: None declared, A. Rosenwald: None declared, T. Brunner: None declared, H. Wajant Conflict with: H.W. is a consultant of Argen-X BVBA, the developer of the Fn14-specific antibodies used in this study.

P499

Immune Reconstitution of Children Who Developed Chronic Graft Versus Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) has been used for treating children affected by

many hematological disorders. Successful immune reconstitution is important for decreasing post-HSCT complications including infections, relapse, and secondary malignancy, without increasing graft-versus-host disease (GvHD). Here, we aimed to evaluate whether there is a relationship between immune reconstitution and chronic graft versus host disease in children who underwent allogeneic HSCT.

Materials (or patients) and methods: In this study, lymphocyte subgroups were evaluated before HSCT and 1, 3, 6, 12, and 24 months after HSCT. Lymphocyte subgroups of children who developed chronic GvHD (n:13) and those of children who did not develop chronic GvHD (n:68) after allogeneic HSCT were compared. In addition to classical lymphocyte subgroups; activated T lymphocyte subgroups including CD8/57(+), CD8/56(+), CD3/HLA-DR(+), CD4/25(+), CD4/28(+) T lymphocytes were evaluated.

Results: When absolute levels of lymphocyte subgroups were compared in children with and without chronic GvHD, CD3(+) lymphocyte count returned to pre-HSCT levels at 1 month in children with chronic GvHD and at 6 months in children without chronic GvHD. CD4(+) T lymphocyte count returned to pre-HSCT levels at 12 months both in children with and without chronic GvHD. While CD8(+) T lymphocyte count returned to pre-HSCT levels as early as 1 month both in children with and without chronic GvHD, CD8(+) T lymphocyte count was higher in children with chronic GvHD at 1, 3, 6, 12, and 24 months when compared to those without chronic GvHD ($P \leq 0.05$). CD19(+) B lymphocyte count returned to pre-HSCT levels at 12 months both in children with and without chronic GvHD, whereas CD19(+) B lymphocyte count was lower in patients with chronic GVHD at 3, 6, and 12 months ($P \leq 0.05$). CD4/8 ratio returned to pre-HSCT levels at 24 months in both groups. CD16/56(+)CD3(+) NK-T and CD16/56(+)CD3(-) NK lymphocytes returned to pre-HSCT levels within 1 month after HSCT in both groups.

When specific subgroups reflecting lymphocyte activation were evaluated in children with chronic GvHD, activated CD8/57(+) T lymphocyte count was higher at 1, 3, 6, 12, and 24 months, while activated CD8/56(+) NK lymphocyte count was higher at 12 and 24 months than those of children without chronic GvHD ($P \leq 0.05$). Activated CD3/HLA-DR(+) T lymphocyte count was higher at 1, 6, 12, and 24 months in the chronic GVHD group. Similarly, CD4/25(+) activated T lymphocyte count was higher at 1, 3, 6, and 24 months in children with chronic GvHD ($P \leq 0.05$).

Conclusion: In conclusion; higher counts of CD3(+) lymphocytes, CD8(+) T lymphocytes, CD8/56(+), CD8/57(+), CD3/DR(+), CD4/25(+) activated T lymphocytes in children with chronic GvHD starting from early stages of HSCT and persisting throughout the follow up period, suggest that these lymphocyte subgroups may play a role in GvHD pathogenesis. Besides, high levels of CD3(+) lymphocytes, CD8(+) T lymphocytes, CD8/57(+), CD3/DR(+), CD4/25(+) activated T lymphocytes at 1 month when chronic GvHD symptoms have not yet developed, suggests that increased numbers of these activated T lymphocytes may be considered as a predictive marker for development of chronic GvHD in children.

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Disclosure of Interest: None declared.

P500

Immunosuppression By Mesenchymal Stromal Cells Derived from Human Induced Pluripotent Stem Cells: Evaluation in an aGVHD Model

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Introduction: Background: One major problem of allogeneic hematopoietic stem cell transplantation is acute Graft versus Host Disease (aGVHD). aGVHD has been managed until now with HLA matching and a constant evolving repertoire of immunosuppressive drugs. One alternative would be to generate in the host a permanent tolerance state toward the graft. Tolerant inducing cell therapy has been proposed with adult mesenchymal stromal (MSCs) cells, such *ex vivo* isolated MSCs displaying immunoregulatory functions on cells from both the innate and adaptive immune system. Nevertheless, their use is restricted because of the few number that can be recovered from adult tissues, their limited *in vitro* expansion, and the absence of a full characterization.Therefore other sources of well-defined and unlimited number of MSCs are needed, and MSCs derived *in vitro* from human Induced pluripotent stem cell (huIPS) would be a valuable tool for therapeutic approaches.

Aims: Thanks to our expertise in pluripotent cell differentiation, we generated huiPS-MSCs. Our objectives are: 1/To evaluate and characterize *in vitro* their immunosuppressive activity. 2/To validate *in vivo* these results using a xenoGVHD model.

Materials (or patients) and methods: To characterize the huIPS-MSCs *in vitro*, FACS phenotyping and multipotency were tested. Their immunogenicity *in vitro* was monitored in co-cultures with allogenic peripheral blood mononuclear cells (PBMC). The *in vivo* immunosuppressive activity of huiPS-MSCs was evaluated using a xenoGVHD model in immunodeficient NOD/SCID/IL2rγKO mice injected intra-peritonally with human PBMC and treated or not by 3 weekly injections of huIPS-MSCs.

Results: a) In vitro characterization of huIPS-MSCs

As expected, the huiPS-MSCs were positive for CD73, CD90, CD105, HLA-I Ags and negative for CD45, CD34, HLA-II Ags and they were capable of differentiation into osteoblast, chondrocytes and adipocytes. The allogenic stimulation of PBMC in mixed lymphocyte reaction resulting in CD4 and CD8 T cell proliferation (28 ± 7% and 47 ± 8%, respectively) was significantly reduced in co-culture with huiPS-MSCs (4 ± 2% and 10 ± 2, respectively, $n = 3$ $P < 0{,}05$). We demonstrated using blocking antibodies that part of the inhibition exerted by the iPS-MSCs is due to a) B7H1, a membrane receptor for the B7 family, known for its inhibitory action on the activation of T cell b) and B7H3 (role remains controversial).

b) *In vivo* characterization of huIPS-MSCs

After sacrifice of mice (5 to 8 weeks after the initial injection), the numbers of human circulating T lymphocytes, of those present in the peritoneal cavity and in the spleen were significantly reduced in mice treated with huIPS-MSCs $P < 0{,}05$. Intracytoplasmic labelling of recovered T cells showed that untreated mice displayed high percentages of differentiated T cells producing IFN and TNF (typical of an inflammatory Th1 profile).In contrast, in mice treated with the huiPS-MSCs, the proportion of T cells of the Th1 type was substantially reduced, while that of T cells producing the anti-inflammatory cytokine IL-10 was slightly increased. In parallel, T cells expressing FoxP3 appeared.

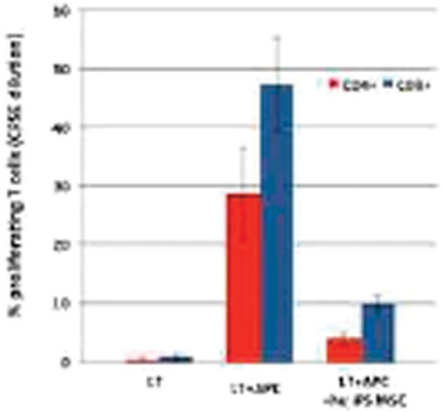

Figure 1 : huiPS-MSCs inhibit proliferation of T cells in vitro

Conclusion: We were able to generate immune-modulatory huiPS-MSCs that can be used to alter activation of T cells in a xeno-aGVHD model. Our results may favor the development of new tolerogenic tools based on the use of pluripotent stem cell derivatives to prevent aGVHD.
Disclosure of Interest: None declared.

P501
Evaluation of T regs and mature T regs in acute and chronic graft versus host disease

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Introduction: Acute and chronic graft versus host disease (GvHD) remains a major complication after allogeneic peripheral stem cell transplantation (alloPBSCT) and many studies have investigated the role of regulatory T cells on acute and chronic GvHD. As regards human T regs, recent studies have shown that T regs are not homogeneous as to immunosuppression function. In this study we evaluated the total T regs ($CD4+,CD25^{high},CD127^{low}$) and mature T regs ($CD4+$, $CD25^{high},CD127^{low,}$ $CD45RA^{neg}$) in peripheral blood in patients with or without acute or chronic GvHD after myeloablative alloPBSCT and the factors (T regs allograft dose, matched related or unrelated donor, CMV serological status of donor and recipient, donor/recipient sex mismatching) that may have an impact on T regs early expansion at 30 days after alloPBSCT.
Materials (or patients) and methods: We evaluated the recovery of total T regs and mature T regs in peripheral blood of 40 patients who never developed GvHD ($n=10$), patients who developed aGvHD grade II-IV ($n=18$) and patients who developed cGvHD ($n=12$). All patients (median age 39 yy) were transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor ($n=31$) or an HLA unrelated donor (HLA 8/8)($n=9$) after myeloablative conditioning regimen; diagnoses were acute myeloid leukemia($n=32$), acute lymphoblastic leukemia ($n=8$). GvHD prophylaxis consisted of cyclosporin A and MTX.
Results: Total T reg were significantly higher in patients without as compared with aGvHD (at onset of GvHD or between 7 days from the onset): 22 (range 6-78) vs 8 μl (range 1-11), respectively ($P<0.001$). In patients with or without aGvHD the median count of mature T regs were 0.5 (range 0-0.7) and 2 μl (0.2-4.8)($P=0.05$), respectively. In patients with cGvHD the median count of mature T regs was 23 μl(range 17-43). The median ratio of total T regs/CD4 was 8%, 1% and 7% in patients without GvHD, with aGvHD and with cGvHD, respectively. Reconstitution of total T regs at 30 days after SCT was significantly better in patients with a matched related donor than unrelated donor transplant ($P=0.004$) and with a T regs allograft greater than 5 x 10^6/kg b.w. ($P=0.003$). In our experience, CMV serological status and donor/recipient sex mismatching did not influence T regs reconstitution.
Conclusion: These results indicate that adoptive transfer of T regs cells may have a clinical impact in the setting of aGvHD but not in cGvHD, in which functional T regs cells are already present despite ongoing disease activity.
Disclosure of Interest: None declared.

P502
Phenotypic and functional stability of regulatory T cells expanded after alloanergization with costimulatory molecule blockade

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Introduction: Induction of alloantigen-specific hyporesponsiveness by allostimulation and costimulation blockade (alloanergization) is an approach to selectively reduce donor T cell alloreactivity to prevent severe Graft-versus-Host Disease (GvHD) after HLA-mismatched allogeneic hematopoietic cell transplantation (AHCT). We and others have shown that alloanergization also results in expansion of donor $CD4^+$ regulatory T cells (Tregs) which potently suppress donor alloresponses *in vitro*. It is now understood that stable phenotype and function is key to suppressive capacity of Tregs, especially in proinflammatory conditions often encountered after AHCT. We therefore characterized Tregs expanded after alloanergization in the presence of proinflammatory conditions to address their stability of suppressive phenotype and function.
Materials (or patients) and methods: Human peripheral blood mononuclear cells (PBMCs) were alloanergized with irradiated HLA-mismatched stimulator PBMCs from unrelated healthy donors and specific blockade of B7.1 and B7.2 costimulatory molecules. Phenotyping and cytokine profiling was performed by flow cytometry and intracellular staining for Interferon (IFN)-γ and Interleukin (IL)-17 in the presence or absence of proinflammatory stimuli (lipopolysaccharide (LPS) or IL-1β/IL-6). Methylation of the Treg-specific demethylated region (TSDR) was quantified using bisulfite sequencing. Treg function was assessed by measuring suppression of first-party proliferative alloresponses.
Results: The proportion of $CD4^+$ cells with a $CD25^+CD127^-$ Treg phenotype within donor T cell pool increased from a median 3.4% at baseline to 4.7%, after alloanergization and 8.9% after subsequent allorestimulation. Although $CD4^+$ Tregs showed progressive TSDR methylation after alloanergization and subsequent allorestimulation, purified Tregs maintained similar expression levels of FOXP3, CTLA-4 and CD39 and displayed potent allosuppressive capacity even under proinflammatory conditions. Importantly, we observed an increase in the frequency of $CD4^+FOXP3^+$ Tregs able to produce IFN-γ.This effect was further enhanced in the presence of LPS or IL-1β/IL-6 without a significant change in the frequency of IL-17^+ $CD4^+$ Tregs. Furthermore, purified IFN-γ^+ Tregs were potent allosuppressors. Finally, although $CD4^+$ Tregs retained expression of CCR7, CD62L and CCR4, expression of CCR9, was progressively reduced following alloanergization and allorestimulation.
Conclusion: Allorestimulated Tregs maintain suppressive phenotype and function even in proinflammatory conditions. Allorestimulation of PBMCs after alloanergization resulted in expanded subpopulations of an IFN-γ^+ Treg subset which may contribute to the maintenance of suppressive function in this context. However, expanded Tregs lost CCR9 expression, suggesting limited ability to migrate to the small intestine, in keeping with the preponderance of gut GvHD observed in patients receiving alloanergized bone marrow transplants in prior clinical studies. These findings provide mechanistic insight into a potential strategy to control alloresponses after AHCT. Approaches to improve the gut-specific migratory capacity of Tregs expanded in this setting may improve the clinical efficacy of the approach.
Disclosure of Interest: None declared.

P503
Treg treated dendritic cells are defective in initiating cutaneous GVH reactions

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Introduction: Early stage clinical trials have reported promising results showing that adoptive transfer of regulatory T cells (Treg) ameliorates graft-versus-host disease (GvHD). However, the mechanisms of Treg mediated protection against GvHD are yet to be fully defined. Dendritic cells (DC) are pivotal in initiating allo-reactive immune responses and are critical in GvHD pathogenesis. This study investigated the phenotypic profile and allo-reactive functions of Treg treated DCs,

particularly the ability of Treg treated DC to induce GvHD target tissue damage.

Materials (or patients) and methods: Immature, mature and Treg treated DCs were generated from immuno-magnetic isolated monocytes (im-DC, mat-DC and Treg-DC respectively). The three moDC populations were generated using the established 6 day culture with GM-CSF and IL-4 followed by 24 h LPS maturation. Treg were added on day 3 of moDC culture. Im-DC, mat-DC and Treg-DC were harvested on day 7, prior to functional assays Treg-DC were isolated by FACS sorting via FSC/SSC/$CD3^-$ gating to remove Treg present in the co-culture.

Results: Using an *in vitro* human GvHD skin explant model we revealed that allo-reactive CD8 T cells primed with Treg-DC had diminished ability to induce cutaneous GvH reactions. Treg-DC induced grade I/II damage, whereas both im-DC and mat-DC induced significantly higher grade III damage in the majority of cases ($P = 0.038$). Interestingly the presence of Treg throughout the allo-response induction resulted in the lowest level of GvH damage; only grade I. These observations were supported by the markedly defective ability of Treg-DC to stimulate activation and proliferation of allo-reactive CD8 T cells, detected by CD25 expression and CFSE dilution respectively ($P = 0.009$, $P = 0.046$). Again the presence of Treg throughout the entire allo-stimulation resulted in a more potent reduction in activation and proliferation ($P = 0.009$, $P = 0.0085$). Further investigation revealed a significant decrease in antigen-capture capacity of Treg treated im-DC compared to untreated im-DC ($P = 0.047$). Additionally Treg-DC displayed a semi-mature phenotype with reduced expression of co-stimulatory molecules CD80/CD86, with expression significantly lower than mat-DC ($P < 0.005$) but significantly higher than im-DC ($P < 0.05$). Treg-DC did express levels of HLA-DR comparable to mat-DC ($P = 0.6357$) allowing them to engage with the T cells. Treg-DC also expressed increased markers associated with tolerance including CCR7 expression comparable to that of im-DC but markedly higher than mat-DC ($P < 0.05$) and significantly higher expression of LAP-TGFβ1 on Treg-DC when compared to that of mat-DC ($P < 0.01$). Furthermore Treg-DC drove an altered polarisation of naïve CD4 T cells, as determined by cytokine expression, secretion and gene expression. Polarisation by Treg-DC resulted in a skewing away from Th1 cells towards Treg cells which may contribute to the ability of Treg to control GvH responses *in vivo*.

Conclusion: In conclusion, attenuation of DC signature and function is key for Treg mediated protection against GvHD. However, isolated DC modulation by Treg was less effective in suppressing CD8 T cell allo-responses compared to the continued presence of Treg during the CD8 T cell priming, activation and proliferation, suggesting that Treg exert most effective function via multi-dimensional modulation on the DC, T cells, DC-T cell interactions and naïve T cell polarisation simultaneously.

Disclosure of Interest: None declared.

P504

MyD88 in donor bone marrow cells is critical for protection from acute intestinal graft-versus-host disease

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Introduction: Development of graft-versus-host disease (GVHD) after hemopoietic stem cell transplantation causes non-relapse mortality and substantial morbidity of recipients. Myeloid differentiation factor 88 (MyD88), a major adaptor mediating TLR signaling, is also known to deliver pro-inflammatory signals. Activation of inflammatory signaling through MyD88 plays a key role in the expansion of myeloid-derived suppressor cells (MDSC) which are a heterogeneous population of immature myeloid ells with anti-inflammatory activity.

Materials (or patients) and methods: To explore the contribution of MyD88 expressed by donor bone marrow (BM) cells to development of GVHD, we induced GVHD using T-cell-depleted BM (TCD-BM) isolated from MyD88-deficient (MyD88KO) mice and T cells isolated from wild-type (WT) mice. We employed C57BL/6 (H-2^b) $\rightarrow$ B6D2F1 (H-$2^{b/d}$) mouse model of GVHD, which differ at major and minor histocompatibility loci. Lethally irradiated B6D2F1 recipient mice were transplanted with either TCD-BM (5×10^6) from either WT or MyD88KO mice together with WT spleen T cells (1×10^6).

Results: Transplantation with MyD88KO TCD BM aggravated GVHD; serious gut damage was evident, with infiltration of T cells specifically into the intestines of recipients. GVHD hosts transplanted with MyD88KO TCD BM exhibited markedly reduced expansion of MDSC. GVHD aggravation after transplantation with MyD88KO TCD-BM, associated with high-level T cell infiltration into the intestine and insignificant expansion of MDSC, was reproduced in another minor histocompatibility mismatch model (C57BL/6 $\rightarrow$ BALB.B).

Conclusion: The results of our study afford an understanding of the mechanism by which MyD88-mediated signaling in donor BM attenuates the severity of GVHD, and represents a critical step toward the potential clinical application of MDSC.

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Disclosure of Interest: None declared.

P505

Abstract Withdrawn

P506

Developing an *in vitro* predictive test for steroid response in graft versus host disease

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Introduction: A limitation to treatment success of haematopoietic stem cell transplantation (HSCT) is the occurrence of acute and chronic graft-versus-host-disease (GvHD), which manifests in 30-60% allogeneic-HSCT recipients. The initial therapy for GvHD is steroids, which suppress the immune system and reduce inflammation. However, steroids have a variable response rate (35-70%) and for those steroid-refractory patients, second-line treatment is delayed which may significantly impact outcome. A predictive test or biomarker for steroid response would allow for an alternative immunosuppressive therapy to be used as an immediate front-line therapy.

Materials (or patients) and methods: We have used the well-established human *in vitro* GvHD skin explant model to examine the feasibility of predicting steroid response to methylprednisolone (Mpred) ($N = 14$) and Cyclosporin A (CsA) ($N = 6$) using the extent of skin damage, graded from I-IV in severity, as a readout of treatment response. In brief, mixed lymphocyte reactions were set up using HLA-mismatched, irradiated auto-HSCT patient peripheral blood mononuclear cells (PBMCs) as stimulator and PBMCs from healthy volunteers as responder. Following seven days incubation, MLR primed responder cells were co-cultured with skin biopsies autologous to the stimulator, with the addition of Mpred (100 ug/ml) or

CsA (200ng/ml). Primed responders cells and medium alone were used as a positive and negative control, respectively. After a further three days of culture the skin explants were histopathologically graded according to Lerner criteria.
Results: Those samples that were dosed with 100 ug/ml Mpred had a variable histopathological grade: 36% were scored at grade I, 21% grade II and 43% at grade III, which may be indicative of a steroid-sensitive, intermediate and steroid-resistant phenotype, respectively. In contrast, addition of 200 ng/ml CsA to the skin co-culture did not reduce the severity of skin GvH reaction with all 6 samples assessed having a histopathological grade of III.
Conclusion: In conclusion, we have investigated the feasibility of using the skin explant model as a possible *in vitro* predictive test for *in vivo* steroid response and show that it can identify differences in steroid-response as reflected in the variable histopathological grade seen in the pilot cohort used in this study. Future work will involve using a dose range of Mpred to assess the level of sensitivity of the assay and extending the cohort to include HLA matched patient/donor pairs which will be correlated to the clinical outcome of GvHD patient response.
Disclosure of Interest: None declared.

P507
Abstract Withdrawn

P508
Improving the therapeutical effectiveness of extracorporeal photopheresis by using new photochemotherapeutic compounds

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Introduction: Extracorporeal photopheresis (ECP) is a clinically used cell-based immunomodulatory therapy that involves exposure of peripheral blood autologous mononuclear cells to the photosensitizer 8-methoxypsoralen (8-MOP) and UVA radiation before reinfusion. ECP has demostrated efficacy in the treatment of multiple diseases such as graft-versus-host disease (GvHD). During UVA irradiation, 8-MOP covalently binds to DNA, inducing apoptosis. It has been previously reported that 8-MOP-mediated apoptosis triggers some immumodulatory effects that finally lead to the acquisition of peripheral immunotolerance. This mechanism accounts for the clinical benefit of this therapy. In the present work the *in vitro* activity and immunomodulatory effects mediated by 8-MOP in comparison with other two new photosensitizer (BB01 and BB02) has been analyzed, aiming to find novel more effective photochemotherapeutic compounds to use in ECP. Moreover, the therapeutic effectiveness of BB01 and BB02 by ECP was also evaluated in an experimental murine model of GvHD.
Materials (or patients) and methods: Human mononuclear cells (MNC) were incubated with increasing concentrations of 8-MOP, BB01 or BB02, and irradiated with UVA light. The MNC apoptosis percentage was measured by flow cytometry after 48 h. Also, mixed lymphocyte cultures (MLC) with either myeloid immature (iDC) or mature dendritic cells (mDC) and MNC treated with the different compounds and UVA were performed. After, we studied: 1) CCL21-stimulated migration of iDC/mDC, 2) proliferation of treated MNC, 3) production of pro- and anti-inflammatory cytokines, 4) generation of tolerogenic DC and 5) differentiation of Treg. Also, murine GvHD was induced after transplanting bone marrow cells and splenocytes from donor Balb/c mice into C57Bl/6 J recipients. For doing the ECP procedure, splenocytes from separate cohorts of C57Bl/6 J with acute GvHD were isolated, incubated with the different compounds and injected intravenously once a week for four weeks. Survival after transplantation was monitored daily and clinical GvHD was graded using a previously described score analyzing weight loss, posture, activity, skin integrity and fur texture.
Results: There was a significative increase of MNC apoptosis when using BB02 (from 50 ng/ml) and BB01 (from 200 ng/ml) compared to 8-MOP, as well as a significant upregulation of CCL21-promoted migration of iDC and lower migration of mDC, mainly with BB02. Also, MNC proliferation after MLC with iDC or mDC was partially inhibited after treatment with these compounds, together with a significative reduction of levels of pro-inflammatory cytokines and increase of anti-inflammatory IL-10 and TGF-β. Expression profile of DC's maturation and co-stimulatory/MHC-II molecules, as well as modulation in CCR7 expression after MLC demonstrated a more tolerogenic phenotype as well as a more efficient induction of autologous Treg. On the other hand, mice treated weekly with BB02 showed a significant higher survival than those treated with BB01 or 8-MOP. Also, mice treated with either compound improved their clinical GvHD score compared to untreated mice group, being significantly lower with BB02 than with BB01 and 8-MOP.
Conclusion: BB01 and specially BB02 showed higher *in vitro* activity and *in vivo* effectiveness and could be considered as a possible better therapeutic alternative than 8-MOP.
Disclosure of Interest: None declared.

P509
Ex vivo lymphocyte TLR7 tolerance induction effectively prevents murine acute graft-versus-host disease

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Introduction: Acute graft-versus-host disease (aGvHD) is a life-threatening side-effect of allogeneic hematopoietic cell transplantation (allo-HCT) that limits its effectiveness. Conventional anti-GvHD prophylaxis and treatment is associated with generalized immunosuppression and increased susceptibility to opportunistic infections. Toll-like receptors (TLRs) are innate immune receptors recognizing pathogen-derived products and endogenous self-antigens. Whereas TLRs activate immune cells, regulatory mechanisms of TLR tolerance induction have recently been recognized in the context of autoimmuntity. In order to address whether TLR tolerance induction could prevent allo-reactivity and aGvHD using a clinically applicable approach, we investigated the ex-vivo TLR tolerance induction to donor lymphocytes.
Materials (or patients) and methods: The *in vitro* TLR2,4,7,9 tolerance induction was tested by 1-3 day exposure of mouse splenocytes (mSPLCs) to repeated low-doses of TLR-specific agonists, followed by a high-dose challenge.The optimal dose/duration for each agonist to induce maximum hyporesponsiveness was determined by TNF-α quantification (ELISA) in mSPLCs culture supernatants.A non-specific high-dose challenge was used to check for cross-tolerance among TLRs.The capacity of the ex-vivo TLR-tolerized lymphocytes to prevent aGvHD was tested in a fully mismatched transplantation mouse model. Balb/c irradiated recipients received from C57BL/6 donors T-cell depleted bone marrow cells (TCD-BM) alone or with mSPLCs (Groups I, II,) and TCD-BM + mSPLCs pretreated with Pam3CKS4, LPS and R848 (Groups III,IV and V).The status of ''tolerance'' of the transplanted mSPCs was

confirmed before transplantation by high-dose specific challenge. The clinical assessment of aGvHD was based on a 10-point murine GvHD scoring system.
Results: Maximum *in vitro* TLR2-,4-,7-, but not TLR9-, tolerance towards a subsequent challenge with the specific stimulus was induced by a 3 day-exposure of mSPCs to Pam3CSK4,LPS,R848 ($P<0.001$) and CpG-ODN respectively, and the optimal "desensitization" dose for each agonist was defined. TLR-ligands induced specific TLR tolerance of mSPCs but also produced a strong cross-tolerance effect towards the other TLRs. In the mismatched transplantation model, all animals from Groups II,III succumbed before day 29 while $<10\%$ of Group IV recipients survived until day 60 post-transplantation. In contrast, Group V recipients presented significantly less aGvHD ($P<0.001$) and weight loss ($P<0.05$) whereas they were surviving at higher rates ($P<0.001$) at the end of the experiments. In experiments with exvivo R848-pretreated purified T-cells, successful control of aGvHD was demonstrated again over the differently "desensitized" T-cells. Upon sacrifice, histopathology of target tissues demonstrated severe aGvHD lesions in Groups II,III, IV and non specific findings in Groups I,V.The control of aGvHD in group V, over the groups II-IV, was associated with significantly higher IFNγ mRNA levels in PBMCs and a shift towards a T-reg phenotype of blood cell-s($P<0.05$).Interestingly, R848-challenged mSPLCs from day20 sacrificed animals demonstrated strong hyporesponsiveness as opposed to normal TLR activation of mSPLCs from the rest of groups, suggesting continuing tolerance.
Conclusion: The ex-vivo, TLR7-specific, tolerance induction in donor lymphocytes could serve as a clinically applicable and effective tool for aGvHD prophylaxis.
Disclosure of Interest: None declared.

P510
Abstract Withdrawn

P511
A free radical scavenger NecroX-7 attenuates graft-versus-host disease by reciprocal regulation of Th1/Treg and inhibition of HMGB1 release

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Introduction: Graft-versus-host disease (GVHD) is a major complication associated with allogeneic hematopoietic stem cell transplantation (HSCT). Despite the prominent role of the adaptive immune system, the importance of controlling the innate immune system in the pathogenesis of GVHD has recently been rediscovered. High-mobility group box 1 (HMGB1) is a crucial damage-associated molecular pattern (DAMP) signal, which functions as a potent innate immune mediator in GVHD. In the present study, we investigated treatment of experimental GVHD through HMGB1 blockade using the compound NecroX-7.
Materials (or patients) and methods: Therefore, we established a protocol for evaluating the NecroX-7 efficacy on GVHD treatment using a mouse major MHC-mismatched C57B6 (B6)→BALB/c (B/c) GVHD model system. Following transplantation, recipients were given NecroX-7 injections in 2 day intervals for 2 weeks. All animals were monitored for survival and clinical signs of GVHD.
Results: Treated animals significantly attenuated GVHD-related mortality and inhibited severe tissue damage. These protective effects correlated with the decrease in HMGB1 expression and lower levels of reactive oxidative stress (ROS). In addition, NecroX-7 inhibited the HMGB1-induced release of tumor necrosis factor and interleukin-6, as well as the expression of Toll-like receptor-4 and receptor for advanced glycation end products. We also observed increased regulatory T cell (Tregs) numbers, which may be associated with regulation of differentiation signals independent of HMGB1. Taken together, these data indicate that NecroX-7 protects mice against lethal GVHD by reciprocal regulation of Treg/Th1, attenuating systemic HMGB1 accumulation, and inhibiting HMGB1-mediated inflammatory response.
Conclusion: Our results indicate the possibility of a new use for clinical drug that is effective for the treatment of GVHD.
Disclosure of Interest: None declared.

P512
Common Protein Changes in Target Organs during Acute Graft-Versus-Host Disease

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Introduction: To gain additional knowledge on mechanisms contributing to GVHD, we studied protein expression changes in target organs during acute GVHD.
Materials (or patients) and methods: To simulate the clinical situation of patients undergoing MHC matched allo-HSCT, we used Bu/Cy conditioning in the minor MHC mismatched LP/J→C57BL/6 murine model. At maximum of acute GVHD (day + 16 to day + 23 after BMT) liver, colon and skin were harvested and total proteins were isolated. Quantification data of labeled peptides were measured considering N-termini and lysine dimethylation on light (+28 Da), medium (+32 Da) or on heavy (+36 Da) modification per free primary amine. Protein and peptide quantitation information were extracted from MaxQuant 1.2.2.5.
Results: In total, between 2238-4333 proteins and 14489-32562 peptides per group were quantified by dimethylation labeling. Compared to the syn-BMT recipients, most over-expressed proteins in allo-BMT recipients were enriched in three KEGG pathways. We found that most proteins that were overexpressed in allo-BMT recipients vs. syn-BMT recipients belonged to one of the following pathways: 1) "antigen processing and presentation"(Figure 1); 2) "natural killer cell mediated cytotoxicity" and 3) "Leishmaniasis".
On the other hand we found in target organs of allo-BMT recipients, that some proteins were significantly down regulated (Table 1), which were enriched in metabolic KEGG pathways and the pathways regulating of protein functions. This reflected that the amino acid, carbohydrate, lipid metabolisms were impaired and the protein translation, exporting, folding, sorting and degradation functions were all disturbed in target organs in allo-BMT recipients during acute GVHD.

Table 1. Down regulated proteins in target organs of allo-BMT recipients in acute GVHD

Proteins	*Liver*		*Colon*		*Skin*	
	day + 16	*day + 23*	*day + 16*	*day + 23*	*day + 16*	*day + 23*
Acot	Acot1, 2, 3	Acot1; Acot13			Acot1	
Psmb5	Psmb5		Psmb5	Psmb5	Psmb5	
Acaa1	Acaa1	Acaa1			Acaa1	
Clec3b	Clec3b			Clec3b	Clec3b	
Ces6	Ces6	Ces6	Ces6			
Was				Was	Was	Was
Aldh		Aldh5a1	Aldh1b1	Adh1	Aldh3a1	Aldh1b1

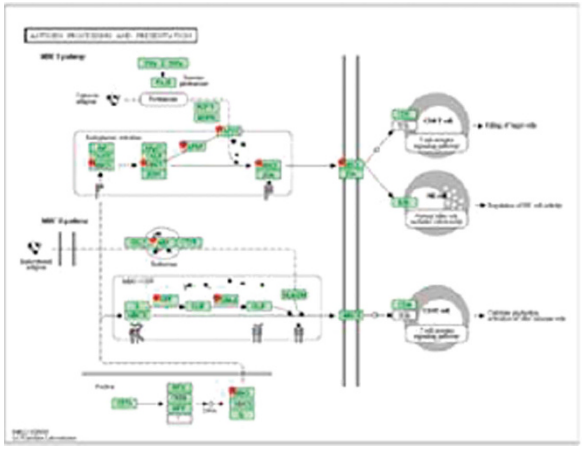

Figure 1: The antigen processing and presentation pathway was activated in target organs of allo-BMT recipients: Compared to the syn-BMT recipients, some overexpressed proteins were enriched in the antigen processing and presentation pathway, which was found in all the allogeneic target organs, except in the liver on day + 23. Red star shows the overexpressed proteins. 4-5 mice per group.

Conclusion: We conclude that protein changes during GVHD are clustered in certain pathways. This knowledge may help identifying yet unknown mechanisms contributing to acute GVHD.

Disclosure of Interest: None declared.

Graft-versus-host disease – clinical II

P513

Early withdrawal of mycophenolate mofetil used as GVHD prophylaxis is associated with worse outcome

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Introduction: Mycophenolate mofetil (MMF) combined with a calcineurin inhibitor in an option of GvHD prophylaxis in allo-SCT. However, nowadays, it is not well established how the first levels can affect the OS and when it has to be stopped. The aim of this study is to evaluate the influence of the levels of MMF measured during the first two weeks post SCT and treatment duration of MMF in graft failure, severe acute GVHD and overall survival (OS).

Materials (or patients) and methods: We retrospectively analyzed 179 patients who underwent an allo-SCT from August 2002 to December 2013 and received MMF as GVHD prophylaxis. MMF was administered from day + 1 of transplant (15 mg/kg/12 h po). We evaluate all the MMF determinations performed during the first 2 weeks (therapeutic level 1-4 mg/dl) and the time to stop MMF and cause of withdrawal. Early withdrawal was considered if patient stopped the treatment before day + 30 in myeloablative(MA) SCT or + 50 in non-myeloabative (NM) SCT.

Results: Patientścharacteristis are showed in table 1. Eighty patients (45%) and 123 patients (69%) were in therapeutic range during the first 7 and 14 days post SCT. We did not find differences in incidence of graft failure, III-IV grade acute GVHD or OS between patients who were below the therapeutic level and patients in therapeutic range during the first and second week.Median days of treatment with MMF were 34 (26-48), 31 days in MA Allo-SCT and 40 in NM allo-SCT. Causes of withdrawal were: end of treatment (68%), unacceptable toxicity (15%), GVHD (11%), infection (3%), death (2%) and unable to achieve therapeutic levels (1%). In 65 patients withdrawal was early due to unacceptable toxicity (30%), GvHD (23%) or medical criteria (36%). Though GVHD was the main cause of death (36% vs 23%, $P = 0.122$) in those patients who experienced an early withdrawal of MMF compared to patients with late withdrawal, there was differences in OS between both groups (24,9 months vs 43,0 months, $P < 0.001$). Univariate analysis showed also statistical differences between both groups in underlying disease at transplant ($P = 0.008$) and type of donor ($P = 0.025$), however the multivariate analysis revealed the early withdrawal of MMF as the main risk factor for mortality with HR 2.48 (IC 95% 1.6–3.8).

Variable	n (%)	
Male/Female	103 (57.5) / 76 (42.5)	
Underlying disease	Acute leukemia/MDS	86 (48.0)
	NHL/Hodgkin lymphoma	45 (25.1)
	Multiple mye oma	17 (9.5)
	Other	31 (17.4)
≥ 2 lines of treatment	113 (63.1)	
Complete response at Allo-SCT	88 (49.2)	
Ablative/Non myeloablative	98 (54.7) / 81 (45.3)	
Donor	Matched related donor idéntico	90 (50.3)
	Matched unrelated donor	50 (27.9)
	Mismatched conor	39 (21.8)
Stem cell source	Bone marrow	121 (67.6)
	Peripheral blood	57 (31.8)
	Umbilical cord	3 (0.6)
ATG administration	39 (21.8)	
Calcineurin inhibitor	Cyclosporine	149 (83.2)
	Tacrolimus	30 (16.8)

Conclusion: In our experience, the early withdrawal of MMF is adversely associated in poor outcome regardless of levels achieved during the first two weeks.

Disclosure of Interest: None declared.

P514

Superior progression and failure free survival with extracorporeal photopheresis compared to standard therapy in chronic GVHD: Reanalyses of a prospective study

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Introduction: Response assessment in chronic GVHD (cGVHD) remains a challenge. Recently, failure free survival (FFS) (defined as absence of subsequent treatment, non-relapse mortality [NRM] or recurrence of underlying malignancy) measured at 6 months (m) after intervention has been considered as a surrogate early clinical trial endpoint. The addition of extracorporeal photopheresis (ECP) was compared to standard of care (calcineurin inhibitor plus steroids) in a prospective randomized study (NCT00054613, Flowers, M et al, Blood 2008) with a primary endpoint of impact on skin manifestations at week (wk) 12 measured by total skin score (TSS). We proposed that ECP would be associated with superior FFS and progression-free survival (PFS) in this cohort.

Materials (or patients) and methods: One hundred patients (pts) were randomized in a 1:1 ratio between 6/2002 and 4/2005. ECP was administered for 12 wks, and in responders,

continued through wk 24. In non-responders (defined by TSS) pts on control arm crossed over to ECP. The extension phase of the study monitored outcome from wk 12 through 24. All analyses were done as intention to treat. Original data files were obtained. PFS was defined as worsening in any organ system as determined by the clinical assessor and recorded in the case report form, worsening of liver function tests (as defined by NIH consensus criteria), death, early termination due to serious adverse event, and an increase in skin score by 1 (TSS converted to NIH skin score). 4 pts had missing baseline and were considered as failures. Statistical plans were confirmed with the sponsor prior to starting analyses.

Results: Pt, transplant and cGVHD characteristics of this cohort have been previously published. The incidence of radiation (RT) based regimens was higher in the ECP arm (68% vs. 42%, $P = 0.009$), while the incidence of gastrointestinal (GI) involvement was higher in the control arm (22% vs. 4%, $P = 0.007$). No baseline characteristics were associated with higher incidence of PFS or FFS in either arm. At wk 12, the probability of PFS was 49.5% (95% CI 34.7 to 62.7) in the ECP group, compared with control group (19.7%, 95% CI 9.5 to 32.6) ($P < 0.001$) (Figure 1). Multivariable analysis (MV) is shown in Table 1. FFS analyses included the extension cohort (wk 12-24 for patients that crossed-over, through wk 24 for patients that stayed on ECP). Additional follow up of up to 3 months was available for most patients. 6 m FFS in the ECP and control group was 33.4% (95% CI 20.2-47.1) and 6.5% (95% CI 1.7-16), respectively ($P = 0.0015$) (Figure 2). MV is shown in Table 1.

Variable	*Wk 12 PFS*			*6 month FFS*		
	HR#	*95% CI*	*P*	*HR*	*95% CI*	*P*
Unrelated vs. related donor	1.28	0.72-2.22	0.38	1.05	0.62-1.77	0.86
PBSC vs. marrow	0.56	0.33-0.94	0.02	0.97	0.59-1.58	0.89
No RT vs. RT regimens	0.49	0.28-0.87	0.01	1.46	0.88-2.42	0.14
Steroid refractory vs. dependent	2.02	1.03-3.96	0.04	0.62	0.29-1.3	0.2
GI involvement vs. no GI	2.27	1.13-4.57	0.02	1.23	0.61-2.5	0.5
ECP vs. non-ECP	2.07	1.22-3.5	0.006	2.29	1.4-3.76	0.009

Conclusion: ECP is associated with superior PFS and 6 m FFS in cGVHD. Although these endpoints are subject to treatment and assessment biases, they remain clinically meaningful. Retrospective analyses of these endpoints in this prospective randomized study eliminates bias and strongly suggest that ECP is an effective modality for treatment of steroid refractory or dependent cGVHD.

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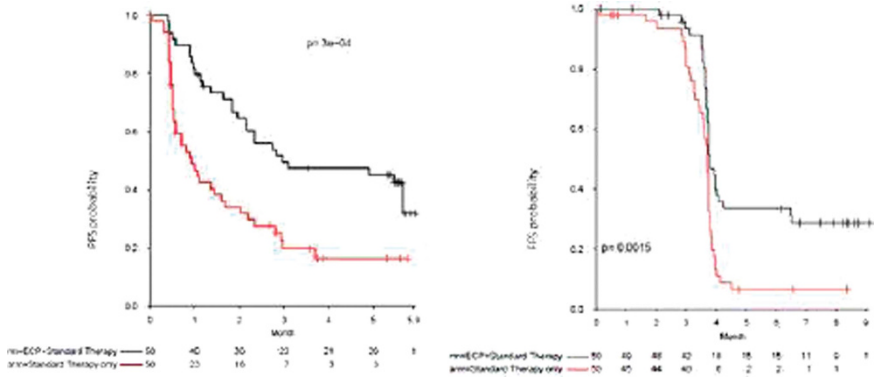
[P514]



P515

The relationship between serum concentrations of interleukin-2 and interferon gamma and acute graft versus host disease after allogeneic hematopoietic stem cells transplantation

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Introduction: The allogeneic Hematopoietic Stem Cells Transplantation (alloHSCT) is associated with the risk of Graft versus Host Disease (GvHD). The patogenesis of acute GvHD is related to T-lymphocytes, which identify alloantigens on host's Antigen Presenting Cells, induce production of interferon (IFN) gamma and interleukin (IL) -2, recruit the immunological effectory cells and destroy tissues and organs.

Materials (or patients) and methods: The study involved 62 patients, 30 (48%) male and 32 (52%) female, aged at median 49.5 (19-68) years, after alloHSCT from sibling ($n = 12$) or from unrelated donor ($n = 50$) performed for acute myeloid leukemia (AML) in Katowice in years 2012-2014. All patients received standard immunosuppressive therapy with Cyclosporin-A and Methotrexate plus pre-transplant anti-thymocyte globulin in unrelated setting. Blood samples were collected pre-transplant before start and after (on day -1) the conditioning therapy, and after alloHSCT on days: $+2$ $+4$, $+6$, $+10$, $+20$, $+30$. The IL-2 and IFN-gamma concentrations in serum were determined with use of ELISA assay.

Results: Patients were divided into 4 groups according to the presence of acute GvHD and infection: group I- patients with neither acute GvHD nor infectious complications, $n = 15$ (24%), group II- patients with infectious complications without acute GvHD, $n = 17$ (27%), group III-patients with acute GvHD without infectious complications, $n = 9$ (15%), and group IV-patients with both acute GvHD and infectious complications, $n = 21$ (34%). Analysis of the IFN-gamma levels showed significantly higher values in group II than in other groups on days $+20$ ($P = 0.014$) and $+30$ ($P = 0.008$). The POST-HOC tests revealed lower levels of IFN-gamma on day $+30$ in group I ($P = 0.039$) and in group IV ($P = 0.017$) as compared to group II. In group III levels of IFN-gamma were not detected. The concentration of IL-2 was undetectable in almost all patients at all studied timepoints.

Conclusion: The high level of IFN-gamma in post-transplantation period is related to infectious complications rather than to acute GvHD.

Disclosure of Interest: None declared.

P516

Graft-Versus-Host Disease after Haploidentical Stem Cell Transplantation in High Risk Haematological Diseases: a 10-Years Evaluation at San Raffaele Scientific Institute

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Introduction: Haematopoietic stem cell transplantation (HCT) is the only curative option for patients (pts) affected by high-risk haematological diseases (HRHD), but the availability of a match donor is still an unmet-medical need. Recently alternative donor transplantations have been broadly exploited, reaching results similar to transplants from a compatible donor. Anyway, whoever the donor is, the most relevant complication remains *Graft-versus-Host Disease* (GvHD).

Materials (or patients) and methods: We evaluated incidence, characterization, treatment and outcome for both acute (a-) and chronic (c-) GvHD in haploidentical (haplo) setting. We applied the NIH consensus criteria to stratify GvHD in routine clinical setting. A population of 257 pts was selected from our Institutional database on the basis of their having an HRHD with indication to allogeneic HCT and having received a HCT from an haplo donor (all consecutive haplo HCT were captured) between Jan 2004 and Dec 2013.

Results: Overall-survival (OS) at 1-year was 46%, with better outcome for pts transplanted in complete remission (CR - p 0.0001). Transplant related mortality was estimated to be 30% at 1-year (infections were the leading cause of death).

The 6-months a-GvHD incidence was 45% - median day of onset 21 post HCT (r8-89). Late-onset a-GvHD was documented in 15 pts. Grade I GvHD was documented in 33 pts (28%), grade II in 44 (37%), grade III-IV in 36 (30%) – 6 not evaluable. Skin was the most frequently involved organ (77%). 105 pts received a 1st line therapy based on high-dose prednisone (2 mg/Kg) and 37/105 completely abrogated the a-GvHD. At a 3-months evaluation, 46% of affected pts showed CR of a-GvHD; mortality rate at the same timepoint was 29%.

C-GvHD affected 69/257 pts and 1-year risk of onset was 25% - median day of presentation 139 post HCT (r40-809); 36/69 pts were off immunosuppressive therapy (52%) at presentation.

According to onset presentation 54% were *de novo*, 25% progressive and 19% quiescent GvHD. 41 pts (60%) presented overlap features.

The most frequent involved organ was skin (grade I-III;53 pts-77%). Skin lesions appeared alone or combined to mouth lesions (35 pts – 51%), liver (23 pts – 33%) or eyes (27 pts – 39%) dysfunctions. The median number of involved organs was 3 (r1-7).

Mild c-GvHD was diagnosed in 10 pts (14%), who received topical therapy. 59 pts – c-GvHD moderate (32, 46%) or severe (27, 39%) - received a systemic treatment.

At a 12-months evaluation, the c-GvHD overall response was 48% (CR 25%) and the mortality rate 36%.

a-GvHD affected pts had a worse outcome ($P=$ 0,068) - Landmark analysis of OS at 3 months after HCT. While overall c-GvHD was not associated with a worse outcome (p ns), overlap c-GvHD was related to worse survival in comparison with classic c-GvHD (p 0.0098).

Conclusion: Haplo HCT is a valid option for pts with HRHD in need of a transplant. GvHD is as manageable after haplo HCT, as in full matched setting. Better knowledge and insight in GvHD are providing advance in improving pts outcome. The NIH-consensus criteria are manageable in daily clinical practice and able to translate in a tailored approach to GvHD with benefit on general outcome. Further advance in the development of specific GvHD biomarkers will provide additional crucial information for management, diagnosis and prognostication in GvHD.

Disclosure of Interest: None declared.

P517

High VEGF serum levels on days +50 and +100 after allogeneic stem cell transplantation predict severe chronic GvHD

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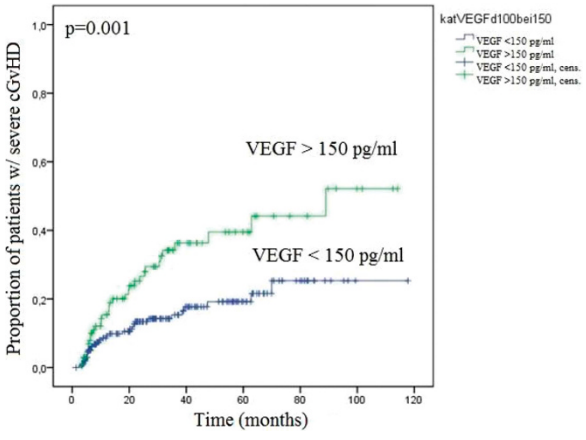
Introduction: Severe chronic graft versus host disease (cGvHD) is one of the main complications following allogeneic stem cell transplantation (SCT). Increasing evidence suggests that endothelial injury and angiogenesis are involved. Chronic GvHD associates with a rarefaction of microvessels in the affected tissue. (Tichelli A. et al. 2008). We therefore hypothesized that VEGF serum levels could be used to predict occurrence of chronic GvHD.

Materials (or patients) and methods: Written informed consent to sample and data collection was obtained from 394 patients undergoing SCT between 2002-2011. Blood serum samples were obtained on day 0, day 50 and day 100. Concentrations of VEGF were quantified by the multiplex protein array technology (Luminex). The occurrence of mild and severe cGvHD was evaluated retrospectively by chart review using the NIH Consensus criteria (Filipovic et al., 2005). The rates of mild and severe cGvHD were plotted using cumulative incidence analysis of cause-specific hazards and compared in various groups using log rank test. Patient characteristics: Median Age: 52 y.o. (17-70), Male/Female 242 (61%)/ 252 (39%), Underlying disease: SAA 5, ALL 42, AML 119, Amyloidosis 1, CLL 29, B-NHL 52, T-NHL 12, CML 15, MPS 19, MDS 32, HD5, MM 61, sarcoma 2, MFD: 148 (38%), MUD 156 (40%), MMUD 90 (23%), MAC/RIC 308 (78%)/86 (22%), ATG/ no ATG 281 (71%)/ 113 (29%), Mild cGvHD166 (42%), Severe cGvHD 75 (19%), Scleroderma or fasciitis 24 (6%), Severe lung cGvHD 17 (4%), Severe GIT cGvHD 26 (7%), Median Time of cGvHD onset 11.18 months (1.4-88.9)

Results: Median serum concentrations of VEGF on days +50 and +100 in those patients developing severe cGvHD were markedly elevated i) d+50: no cGvHD 119.5 (4.3-1577.5) pg/ml, mild cGvHD 113,8 (9.1-620.7) pg/ml, severe cGvHD 158.11 (22.5-415.3) pg/ml; $P=0.044$). ii) d+100: no cGvHD 107.8 (7.8-753.3) pg/l, mild cGvHD 95.4 (15.5-561.9) pg/ml, severe cGvHD 158.1 (20.2-607.2) pg/ml, $P=0.048$). High serum concentrations of VEGF did not correlate with acute GvHD of



any grade. Serum concentrations of VEGF >150 pg/ml on day $+100$ after allogeneic stem cell transplantation were associated with a 2.1 fold higher rate of severe cGvHD ($P=0.001$). **Conclusion:** These results suggest that VEGF serum levels on day $+50$ and day $+100$ after allogeneic stem cell transplantation may be useful for early prediction of severe cGvHD. One explanation for prognostic VEGF elevations occurring long before onset of clinical cGVHD could be that endothelial cell alterations are involved in the pathogenesis of severe cGvHD which develop early but become only relevant after tapering immunosuppression

Disclosure of Interest: None declared.

P518

Elevation of CD19 + CD21low B-cells in patients with chronic graft-versus-host-disease: A validation study in two independent patient cohorts

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Introduction: Chronic graft versus host disease (cGVHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (HCT). Afflicted patients present heterogeneous organ manifestations varying in severity and patients' impairment of quality of life. To date, there is no validated biomarker available that would allow objective diagnosis of active cGVHD. We previously observed a distortion of B-cell homeostasis in patients with cGVHD. In this study we validated B-cell subpopulations including $CD19^+CD21^{low}$ B-cells in the peripheral blood (PB) in two independent patient cohorts after allogeneic HCT as cellular biomarkers for diagnosis of active cGVHD.

Materials (or patients) and methods: At the Medical University of Vienna (MUV) 170 patients with a median age of 40 (range, 18-73) years including 125 with active cGVHD and 45 no-cGVHD were analyzed. Samples were collected within a prospective study on potential biomarkers starting on day 100 after HCT and repeated every 3 months for 2 years. For this validation study a single time point around 9 months after HCT was chosen for analysis. In addition, 25 patients from a cross-sectional study >2 years after HCT were included for comparison with the NIH cohort. Clinical evaluation for cGVHD was performed according to the NIH consensus. Fresh PB whole blood cells were obtained for immunophenotyping and flow cytometry using a Calibur and CANTO II. Most of the patients (81%) had received stem cells from HLA-identical donors and stem cell source was peripheral blood (PBSC) in 91%, respectively. Median duration of cGVHD prior to study enrollment was 4 (range, 0-124) months. The second patient cohort consists of 50 patients with a median age of 50 (range, 15-66) years from the National Institutes of Health (NIH) including 39 with active cGVHD and 11 no-cGVHD. Ninety-eight percent had received stem cells from HLA-identical donors and 88% PBSC. Median duration of cGVHD prior to study enrollment was 28 (range, 0-130) months. In the NIH cohort PB analyses were performed on frozen samples obtained at study enrollment. For flow cytometry the same gating strategy was used in both patient cohorts.

Results: In both patient cohorts percentages of $CD19^+CD21^{low}$ B-cells were significantly higher in patients with active cGVHD (MUV: 27.08 vs. 8.91, $P<0.001$; NIH: 12.09 vs. 5.08, $P=0.024$). Furthermore, a significant elevation in relative numbers of $CD19^+CD21^{low}CD38^{low}$ B-cells (MUV: 11.65 vs. 3.76, $P<0.001$; NIH: 9.11 vs. 2.53, $P=0.003$) and $CD19^+CD21^{low}CD27^-$ B-cells (MUV: 21.69 vs. 7.13, $P<0.001$; NIH: 10.67 vs. 4.27, $P=0.028$) was observed in patients with active cGVHD compared to the no-cGVHD control, respectively. In the MUV patient cohort both in cGVHD patients less than and more than 2 years after HCT significant elevations of these B-cell subpopulations were observed compared to the no-cGVHD control.

Conclusion: In two independent patient cohorts $CD19^+CD21^{low}$ B-cells as well as their subsets were significantly associated with active cGVHD. Absolute and relative numbers of various B-cell subpopulations differed between the two separate institutions due to the fact that clinical patient characteristics and duration of cGVHD differed and fresh versus frozen samples were analyzed. Nevertheless, our results are promising and confirm our previous findings.

Disclosure of Interest: None declared.

P519

Prophylactic Extracorporeal Photopheresis for GVHD prevention after Reduced Intensity Conditioning and Allogeneic Stem Cell Transplantation from Identical Siblings and 10/10 HLA Unrelated Hematopoietic Stem Cells Donors

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Introduction: We performed this prospective multicenter phase 2 study to evaluate the safety and efficacy of prophylactic use of extracorporeal photopheresis (ECP) early after allo-HSCT in patients with hematological malignancies.

Materials (or patients) and methods: Eligible patients were adults with hematological malignancy candidate for allo-HSCT, with controlled disease at transplantation and having a 10/10 HLA identical related or unrelated peripheral blood stem cells (PBSC) donor. All patients must have received a reduced intensity conditioning regimen consisting on Fludarabine (30 mg/m^2/day, from day -5 to day -1), iv. Busulfan (0.8 mg/kg every 6 hours, from day -4 to day -3) and rabbit ATG (2,5 mg/kg, on day -2 and day -1). GVHD prophylaxis consisted on CsA alone, associated to methotrexate in case of major ABO incompatibility. ECP was initiated at day 21 after transplantation, twice per week during the first two weeks and then once per week for the next four weeks, a total of 8 ECP courses had to be performed for each patient. ECP related toxicity and adverse events were evaluated according to NCI/NIH common toxicity criteria up to day 100 after transplantation.

Results: Between June 2009 and May 2014, a total of 20 patients were included; 10 males and 10 females with a median age of 60 years (range: 47-66); 7 (35%) had AML (4 de novo and 3 secondary), 4 (20%) CLL, 3 (15%) NHL, 2 (10%) multiple myeloma, 2 plasma cell leukemia and 2 MDS. At transplantation, 14 (70%) patients were in CR, 2 in VGPR and 4 in PR; all patients received PBSC from 8 (40%) identical siblings and 12 (60%) 10/10 HLA unrelated donors. For sex matching, only 3 (15%) were female donors to male patients. The median number of injected CD34+ cells was 6.10^6/Kg (range: 2.7-10.4). After transplantation, all patients engrafted, 17 (85%) received 8 ECP courses, one patient received 7 courses due to catheter dysfunction, one patient received 5 courses due to severe infection and one patient received only 4 courses due to early death. There were no unexpected adverse effects related to ECP. Among 12 (60%) patients evaluated for chimerism at day 100, all had full donor cells. Seven patients developed acute GVHD, all resolutive, 4 grade I, 1 grade II, 1 grade III and 1 grade IV with a cumulative incidence (CI) at 3 months of 15% (7-23) for grade $\geq$II. Four patients experienced chronic GVHD, 3 limited and 1 extensive all resolutive with a 2 years CI of 22% (13-31). After a median follow-up of 25 months (range: 2-52), the probability of overall survival at 2 years was 84% (range: 75-93), the two years probability of progression-free survival was 74% (range: 65-83), and the cumulative

incidence of transplant-related mortality was 11% (range: 4-18) at two years. At the last follow-up, 14 patients were alive (13 in CR and 1 in relapse) without GVHD, 6 patients died all from relapse (among them 2 were associated with cGVHD after receiving DLI for relapse).
Conclusion: This prospective phase 2 multicenter study demonstrated the safety of prophylactic ECP after allo-HSCT. We showed encouraging results with very low acute and chronic GVHD incidence and no interference with GVL effect in view of the good results in terms of OS and PFS. Larger phase 3 study is now required to validate the benefit of this strategy
Disclosure of Interest: None declared.

Table 1. Patient characteristics

	Test cohort (n=26)	Validation cohort (n=40)
Mean age, years	48 ± 12	52 ± 17
Sex (female/male)	12/14	17/23
Sex-mismatched HSCT	10/26	19/40
Disease (WHO criteria)		
AML with recurrent genetic abnormalities	6	12
AML with myelodysplasia-related changes	7	11
Therapy-related myeloid neoplasms	3	4
Acute myeloid leukemia, not otherwise specified	10	13
Acute GvHD (no/yes)	13/13	20/20
Grading acute GvHD (I/II/III/IV)	4/7/2/0	9/9/2/0
Acute GvHD clinically relevant (≥ grade 2)	9	11
Chronic GvHD (no/yes)	9/17	18/22

Abbreviations: GVHD = graft-versus-host disease; HSCT = hematopoietic stem cell transplantation

P520
Effect of miRNAs and gene variants on GvHD after allogeneic stem cell transplantation for patients with acute myeloid leukemia

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Introduction: Single-nucleotide polymorphisms (SNPs) and microRNAs (miRNAs) are molecular markers that vary significantly among different populations. Our group has earlier reported about genetic associations of SNPs or miRNAs on GvHD after allogeneic hematopoietic stem cell transplantation (HSCT) in different retrospective studies. Here we profiled SNPs and miRNAs in a non-interventional prospective study (Trial No DRKS00004352) about the influence on GvHD for patients (pts) with acute myeloid leukemia who underwent allogeneic HSCT between June 2011 and February 2013.
Materials (or patients) and methods: We analyzed simultaneously 46 different genes of every patient/donor pairs and 6 different miRNAs in whole blood by LightCycler® real-time PCR-system.
Results: In this cohort 16 pts received grafts from HLA-identical siblings (21%), 48 pts from matched (64%) and 11 pts from mismatched (15%) unrelated donors. Transplant consisted of unmanipulated peripheral blood stem cells ($n=72$, 96%) or bone marrow ($n=3$, 4%). Of all pts ($n=75$, male 33 pts and female 42 pts), 20 (27%) had relapsed and 21 (28%) died of April 2014. In the cohort the occurrence of acute GvHD (aGvHD) grade 2-4 was influenced by gene variants on recipient side of CYP2C9 (26% vs 83%, $P<0.00001$), LCT (59% vs 27%, $P<0.04$), and NFκB (80% vs. 20%, $P<0.04$). Furthermore, the occurrence of severe aGvHD ≥ 3 was influenced by CTLA4 (3% vs 20%, $P<0.05$), CYP2C9 (6% vs 27%, $P<0.03$), IL16 (3% vs 19%, $P<0.03$), and NFκB (40% vs 7%, $P<0.03$). In regard to chronic GvHD, we found an influence by gene variants of CYP3A5 (36% vs 83%, $P<0.03$), MDR1 (56% vs 27%, $P<0.04$), NOD2 (50% vs 0%, $P<0.02$) and miRNA-181 (28% vs 55%, $P<0.02$). The estimate 1-year none-relapse mortality (NRM) was associated favorably with the detection of variants of NFκB genes (12% vs 5%, $P<0.05$) and associated adversely with the detection of miRNA-146 (5% vs 18%, $P<0.02$).
Conclusion: These results suggest that different gene variants and/or miRNAs have influence on the transplant settings in pts with acute myeloid leukemia.
Disclosure of Interest: None declared.

P521
Numerical impairment of nestin + bone marrow niches in acute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation for acute myeloid leukaemia

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Introduction: There is increasing evidence suggesting that the perivascular bone marrow stem cell niche and intramedullary neoangiogenesis are involved in GvHD. Nestin, originally described as a marker of neuroepithelial stem/progenitor cells in the central nervous system, is expressed in a variety of undifferentiated tissues under normal and pathological conditions. Cells expressing nestin show all the characteristic features of stem cells – multipotency, self-renewal and regeneration capacity. Further, tissue-resident nestin$^+$ multipotent stem cells seem to be involved in vessel stabilization and the nestin$^+$ perivascular compartment is considered to represent the reticular haematopoietic stem cell niche (HSCN).
Materials (or patients) and methods: To study our hypothesis that GvHD impairs the number (and thus the function) of the nestin$^+$ HSCN, we examined a test cohort of 26 patients with acute myeloid leukaemia (AML), who had undergone allogeneic haematopoietic stem cell transplantation (HSCT) (Table 1). All were in complete remission and had none ($n=13$) or acute GvHD (aGvHD; $n=13$). For result confirmation we examined a validation cohort of 40 AML patients of whom 20 had no aGvHD, 9 suffered from aGvHD grade 1 and 11 had clinically relevant (⩾ grade 2) aGvHD. We performed immunohistochemical studies of the respective bone marrow biopsies 1 month after allo-HSCT using antibodies against nestin, CD34 (for bone marrow microvessel density [MVD] determination), procollagen 1, and FoxP3.
Results: In our test cohort, we found that, despite elevated bone marrow MVD in patients with aGvHD, nestin$^+$ HSCN per mm^2 were markedly reduced compared to patients without aGvHD (1.2 ± 0.78 *versus* 2.6 ± 0.93, $P=0.04$). In our validation cohort we found also an increased MVD in patients with clinically relevant aGvHD (19.5 ± 4.1 *versus* 14.4 ± 5.9 vessels/mm^2; $P=0.007$) whereas nestin$^+$ HSCN were reduced (1.8 ± 0.98 *versus* 2.4 ± 0.97 nestin$^+$ HSCN/mm^2, $P=0.09$). Receiver operating curves and Youden's index suggested a potential discriminatory power of nestin$^+$ HSCN quantities for the set variable aGvHD (AUROC = 0.68, 95% CI 0.513-0.874, $P=0.05$) and a cut-off score best discriminating between patients with and without GvHD of 2.29 nestin$^+$ HSCN/mm^2. Applying this cut-off score 9/11 patients with aGvHD (⩾ grade 2) had decreased nestin$^+$ HSCN numbers compared to only 10/29 patients without aGvHD ($P=0.007$). We found no significant differences regarding FoxP3$^+$ cells per mm^2 and procollagen 1$^+$ osteoblasts in patients with and without aGvHD.
Conclusion: As the bone marrow nestin$^+$ mesenchymal stem cells and the HSCN are innervated and rescued from apoptosis by sympathetic nerve fibres and their number decreases in aGvHD, a therapy with neuroprotective sympathomimetic drugs could be a novel therapeutic option in patients with aGVHD bypassing known barriers related to immunosuppression. Taken together, our results suggest that new vessel formation and numeric impairment of nestin$^+$ HSCN are involved in aGvHD.
Disclosure of Interest: None declared.

P522

Tolerance induction with post-transplantation cyclophosphamide and mesenchymal stem cells infusion for patients with advanced disease after allogeneic bone marrow transplantation

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Introduction: Allogenic bone marrow transplantation (allo-BMT) with high-dose, post-transplantation cyclophosphamide (CY) to promote graft-host tolerance has become an alternative for standard immunosuppression. This post-BMT CY-based immunosuppression regimen has comparable efficacy with other immunosuppressive regimens including cyclosporine A and mycophenolate mofetil. Previously we reported a significant reduction of acute GVHD rate by using MSC infusion for GVHD prophylaxis [ClinicalTrials.gov:NCT01941394]. So we studied high-dose, post-transplantation cyclophosphamide and mesenchymal stem cells (MSC) infusion for graft-versus-host (GVHD) prophylaxis for patients with advanced disease after FLAMSA (amsacrin substituted for idarubicin) and FluBuATG conditioning regimen without standard posttransplant immunosupression.
Materials (or patients) and methods: Since June in 2012 - 21 patients with a median age 38 years (22-59 years; 14 males, 7 females; 17 donors/recipient pairs were sex matched, 4 was sex mismatched) were included. All patients has an advanced disease (relapsed or refractory AML $n=12$ ALL $n=3$, MM progression $n=4$, MPN $n=2$) with a median blast cells 15%, underwent allo-BMT ($n=12$ from HLA-identical related donor, and $n=9$ from unrelated donor). Conditioning regimen was composed of fludarabine, busulfan, and horse anti-thymocyte immunoglobuline for 8 patients, in 11 patients FLAMSA was applied, and 2 patient has treosulfan + cyclophosphamide. For GVHD prophylaxis CY at dose 50 mg/kg daily at day $+3, +4$ was used. All patients also received MSC for acute GVHD prophylaxis at day of recovery (WBC > 1*109/l) at dose 1*106/kg. No other immunosuppression was used.
Results: Neutrophil engraftment was achieved at a median day +24 (range from 13 to 44 days).Acute GVHD occurred in 10 patients (43.5%) at a median day +21; 8 cases were Grade 2-3; 2 patient were Grade 4. There was 2 patients with "classic" ($n=7$) and late refractory GVHD ($n=3$) in this study treated with prednisone at a dose 6 mg/kg, followed by ATG; all other patients responded to initial treatment with cyclosporine A at 3 mg/kg. Overall mortality rate was 43.5%. 1 patient died due VOD (TRM - 4.7%), 2 patients died due infection. 1 patient died from late acute GVHD. 6 patients died due relapse. Relapses occurred in 8 patients (33.3%) only in advanced leukemia patients at a median 2.57 month. No relapse was registered in multiple myeloma group. The overall survival is 52.4% with a median of follow-up 4.55 month. (from 0.2 to 18.5 month)
Conclusion: Despite the fact that we study small group use of cyclophosphamide with MSC for GVHD prophylaxis is a very attractive approach for patients with advanced disease. It's seems to be important that this method is very effective not for refractory acute leukemia patients, but for patients with "slow disease" such is multiple myeloma and lymphoma.
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Disclosure of Interest: None declared.

P523

Analysis of acute graft-versus-host disease clinical features and its risk factors in related HLA-haploidentical high-dose peripheral hematopoietic stem cell transplantation without T-cell depletion *in vitro*

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Introduction: To study the clinical features of acute graft-versus-host disease (aGVHD) and its risk factors in related HLA-haploidentical non T-cell depleted in vitro high-dose peripheral hematopoietic stem cell transplantation (RHNT-PBSCT) we designed.
Materials (or patients) and methods: From July 2002 to December 2012,104 patients received RHNT-PBSCT we designed, using unmanipulated high-dose peripheral hematopoietic stem cell(PBSC),ABU/CY + rATG as conditioning regimen mainly and intensive GVHD prophylaxis regimen included anti-CD25 mAb. We analyzed their aGVHD incidence, location and its risk factors, and compared with that of the 103 patients who taked HLA-matched sibling non T-cell depleted in vitro peripheral blood hematopoietic stem cell transplantation (MSNT-PBSCT) simultaneously.
Results: The cumulative incidence of aGVHD in the related HLA-haploidentical (RH)group was significantly higher than that of the HLA-matched sibling (MS) group [$(56.2 \pm 4.7)\%$ vs $(34 \pm 3.6)\%$,$P<0.05$], but the cumulative incidence of II-IV and III-IV grade aGVHD had no significant difference between the two groups[$(39.5 \pm 2.9)\%$ vs$(21.2 \pm 5.4)\%$, $P>0.05$ and $(12.6 \pm 4.1)\%$ vs $(10.8 \pm 2.4)\%$, $P>0.05$)]; The cumulative incidence of the cutaneous aGVHD was significantly higher in RH group than that in MS group [$(42.3 \pm 3.2)\%$ vs $(17.5 \pm 2.3)\%$, $P<0.05$], the cumulative incidence of liver and gastrointestinal aGVHD between two groups had no significant difference [$(7.7 \pm 2.1)\%$ vs $(12.6 \pm 3.4)\%$, $P>0.05$, and $(16.3 \pm 4.5)\%$ vs (10.3 ± 2.5) %, $P>0.05$]; 3 years disease free survival (DFS) and overall survival (OS) of RH group and MS group were $(63 \pm 5.5)\%$, $(65.2 \pm 4.7)\%$ and $(74.2 \pm 5.4)\%$,$(77.4 \pm 5)\%$ respectively, and there was no significant difference between them ($P=0.078$, $P=0.052$).
Univariate analysis of aGVHD occurrence with HLA haplotype ($P=0.003$) and matched loci($P=0.002$) were significantly correlated. Multivariate analysis showed that only the HLA typing is a risk factor for aGVHD (HR = 1.891, $P=0.03$).
Conclusion: Although the incidence of total aGVHD is higher than that of MSNT-PBSCT, but there is no significant in severe aGVHD. Cutaneous aGVHD is the dominant area, DFS and OS are better. These all indicates that our RHNT-PBSCT is feasible. HLA typing is the only aGVHD risk factor in multivariate analysis.
Disclosure of Interest: None declared.

P524

Reconstitution of T cell subsets and its clinical significance after related HLA-haploidentical high-dose peripheral blood hematopoietic stem cell transplantation without T-cell depletion in vitro

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Introduction: To study the reconstitution and clinical significance of T cells subsets of patients undergoing related HLA-haploidentical non T-cell depleted *in vitro* peripheral blood hematopoietic stem cell transplantation (RHNT-PBSCT) with high-dose PBSC.
Materials (or patients) and methods: A total of 35 patients with 2 or 3 loci HLA-mismatched related donor received RHNT-

PBSCT we designed, using unmanipulated PBSC with mononuclear cells $(14.65 \pm 3.04) \times 10^8$/kg and $CD34^+$ cells $(13.42 \pm 8.43) \times 10^6$/kg, ABU/CY + rATG as conditioning regimen and intensive GVHD prophylaxis regimen included anti-CD25 mAb. The changes in peripheral blood T lymphocyte, regulatory T cells and interleukin-10 (IL-10) in 35 patients and 20 healthy controls (control group) were dynamically monitored by flow cytometry and ELISA.

Results: (1) At 30 days after our RHNT-PBSCT, the percentages of $CD3^+$ and $CD4^+$ T lymphocytes were lower in patients without acute graft-versus-host disease (aGVHD) compared to healthy controls. $CD3^+$ T lymphocytes were recovered to normal levels gradually at 60-90 days after transplantation. The percentage of $CD4^+$ T lymphocytes was significantly lower 30-90 days after transplantation and $CD4^+/CD8^+$ ratio was apparently inverted. However, the proportions of $CD4^+$ $CD25^+$ T cells and $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells recovered quickly in the early stage after transplantation(60-90d). (2) The percentages of $CD3^+$ and $CD4^+$ T lymphocytes were significantly lower in patients with aGVHD than in the control group, but no significant differences were found compared with the patients without aGVHD. However, the numbers of $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells in the aGVHD group were lower than those in the control group and the non-aGVHD group significantly. Especially, the percentage of Treg cells was significantly lower in grades III-IV aGVHD patients than in grades I-II aGVHD patients. (3) Serum IL-10 levels were increased gradually in patients without aGVHD, but still lower than healthy controls dramaticlly. No significant difference in IL-10 levels was detectable between grades I-II aGVHD group and non-aGVHD group in the early stage after transplantation, but significantly lower in the grades III-IV aGVHD group than in the non-aGVHD group. Spearman correlation analysis showed that serum IL-10 levels were negatively correlated with the grade of aGVHD.

Conclusion: The reconstitution of peripheral blood T lymphocyte subsets after our RHNT-PBSCT is better. The decreased percentage of Treg cells maybe increase the risk of aGVHD. Dynamic detection of the number of $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells and serum IL-10 levels in the early stage after our RHNT-PBSCT may predict aGVHD or understand the clinical status in patients. It's similar to other RHNT-HSCT reported.

Disclosure of Interest: None declared.

P525

The mesenchymal stem cells as salvage treatment for refractory acute graft versus host disease in pediatric patients: a Multicenter Survey by the Turkish Pediatric Bone Marrow Transplantation Study Group (TPBMT-SG)

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Introduction: Severe acute graft versus host disease (GvHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation. Mesenchymal stem cells (MSCs) play an important role in endogenous tissue repair. And MSCs possess strong immune-modulatory properties making them a promising tool for the treatment of steroid-refractory GvHD. The objective of this study is to evaluate the treatment results of children with refractory GvHD treated with MSCs.

Materials (or patients) and methods: The outcome of 66 children with GvHD that were transplanted in 9 pediatric centers in Turkey between 2011 and 2014 was retrospectively analyzed. The MSCs were used when the patient did not respond to the first and second step treatment modalities. Sixty six children (17 female, 49 male; median age of 7.5 years, (range 6 months-18 years) all had refractory acute GvHD (grade II-IV) after hematopoietic stem cell transplantation (HSCT) were treated with MSCs in combination with immunosuppressive agents. The MSCs from bone marrow or adipose tissue of HLA-unrelated third-party donors were used at the 35.3 ± 29.7th day after the onset of acute GvHD, at a dose of $1.81 \pm 0.84 \times 10(6)$ cells/kg on first infusion. Fourteen patients received one infusion, 25 patients received two infusions, and 27 patients received three or more infusions with the interval of 15.5 ± 10.3 days between first and second infusion.

Results: One patient (1.5%) with grade II, 22 patients (33.3%) with grade III and 43 patients (65.2%) with grade IV acute GvHD received a total of 168 infusions of MSCs. Organ involvement at baseline was 93.9% gastrointestinal, 89.4% skin, and 39.4% liver. Thirty-tree patients (50.0%) had 2 organs involved, and 23 patients (34.8%) had 3 organs involved. Totally, 27 patients (40.9%) had a complete response (CR) and 29 patients (43.9%) had a partial response (PR, which is determined as one grade reduction) to MSCs treatment, while 10 patients (15.2%) gave no response and had a progressive disease. The observed CR for individual organs was 41.9% for the gastrointestinal system, 59.3% for skin, and 25.9% for liver. None of the patients had side-effects during or immediately after infusions, and no MSCs related tumorigenesis was detected to date through the median follow up period of 195 (range 35-1205) days. The probabilities of overall survival in patients with CR and PR to MSCs treatment were 76.9% and 50.0%, respectively. All 10 patients without response died through the follow up period because of progressive GvHD.

Conclusion: The clinical trials of therapy with MSCs showed promising results in pediatric patients with severe acute GVHD those are resistant to multiple immunosuppressive agents. This strategy may provide a high rate of overall responses of acute GVHD with a low toxicity profile.

Disclosure of Interest: None declared.

P526

Anti-cancer and anti-viral effect of TcR$\alpha\beta(+)$ depleted DLI: Lessons learned from three children and utility for further indications

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Introduction: Alloreactive donor T lymphocytes are pivotal for graft versus leukemia (GvL), however, these active cells can also cause graft versus host disease (GvHD) with a high rate of transplant related mortality. Donor lymphocyte infusion (DLI) is an accepted form of treatment for the relapse or graft failure, and uncontrolled viral infection such as CMV. T cells receptors (TcR) that recognize antigen complex consists of two different protein chains. $\alpha\beta(+)$ T cells that found in most percentage of T cells recognize major histocompatibility complex antigens. Antiviral and antitumoral effects of $\gamma\delta$ $(+)$ T cells have been shown. In this presentation we want to share our experience about the usage of antiviral and anticancer effect of TcR$\alpha\beta(+)$ depleted DLI in three children.

Materials (or patients) and methods: Patient 1: Seven years old boy that had early testicular and medullary relapsed acute lympholastic leukemia (ALL) had experienced greft rejection at $+46^{th}$ day of TcRαβ(+) haploidentical HSCT from her mother. Second haploidentical HSCT was performed from his father (CD34: 14,2x10^6/kg ve TcRαβ(+) 149000 cell/kg). Myeloid, and platelet engraftment were achieved at $+11^{th}$, and $+12^{th}$ day, respectively. Although the course of second transplant was uneventful, he experienced severe CMV infection which was uncontrolled with combinations of ganciclovir, foscarnet, cidofovir and leflunomide. Virus specific donor derived T cell transfer was not available and the PCR of CMV was raised to the 4730000 copy/ml. Three doses of TcRαβ(+) DLI (0,95 x10^6 γδ+ T cells/kg) from the father achieved successfully control of the CMV infection without any GvHD. **Patient 2:** A five months-old boy with T-B-NK+ Severe combined immune deficiency (SCID) origination from RAG-2 mutation had severe respiratory infection. Influenza and Human Boca virus was detected by PCR screening of broncoalveolar lavage. He had TcRαβ(+) haploidentical HSCT from his mother. The clinical respiratory distress was managed with 5 doses of TcRαβ(+) DLI (7,69 x10^6 γδ+ T cells/kg) in a week from the donor. But he died at $+93^{rd}$ day because of acinetobacter sepsis. **Patient 3:** An eleven years old boy with relapsed ALL had allogenic HSCT from his brother but second relapsed was experienced. TcRαβ(+) haploidentical HSCT from her mother was performed (CD34: 11,4x10□/kg ve TCR α/β 26000 cell/kg). Myeloid, and platelet engraftments were both achieved at the $+12^{th}$ day. Although he achieved 100% chimerism at $+28^{th}$ day, the chimerism decreased to 25% at $+66^{th}$ day. He was treated with TcRαβ(+) DLI (1x10^6 γδ+ T cells/kg) from her mother combined with chemotherapy. After first TcRαβ(+) DLI he achieved 45% chimerism and after second TcRαβ(+) DLI he achieved 65% chimerism with experienced grade II skin GvHD, but died because of brain hemorrhage.

Results: TcRαβ(+) DLI's have beneficial effects on viral control and enhancing the anti tumor effect. It is more safe from classic DLI procedure because of depletions of the TcRαβ(+) T lymphocyte.

Conclusion: Although our experience is limited, the results are encouraging. Further studies are necessary to highlight the possible utility of alpha beta depleted DLI in untreatable virus infection and anti-leukemic effect for the children with refractory and relapsed leukemia.

Disclosure of Interest: None declared.

P527

Low incidence of Gastro Intestinal acute GVHD without use any antibacterial decontamination

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Introduction: Intestinal acute GVHD is a severe complication after allogeneic stem cell transplantation (SCT). Commonly antibacterial prophylaxis based of oral nonabsorbable antibiotic such as (neomycin colistin, gentamicin, vancomycin) used before and after engraftment to prevent invasive infection however the exact interaction with gastro intestinal acute GVHD (GI aGVHD) remain unclear.

Recent study from Bacterial 16 S rRNA gene sequences suggest that highly diverse bacterial populations inhabit the gastrointestinal tract can modulate host inflammation and promote immune tolerance and remain an important factors to decrease incidence of GI aGVHD after allogeneic SCT [1].

The objective of this study was to evaluate incidence GI aGVHD in allograft patients when without use any antibacterial digestive decontamination prophylaxis.

Materials (or patients) and methods: A total of 15 evaluable consecutive patients with haematological disease were included in period of February 2013 to August 2014. Conditioning regimen were MAC for 15 patients with malignancies diseases (12 AML,1 ALL) including BU/FU (busulfan 130 mg/m^2/d, one shout for 3 hours, -6d to -3d, iv), fludarabine (40 mg/m^2/d, -6d to -3d, iv), additional to rabbit antithymocyte globulins ATG (2,5 mg/kg/d, -2d to-1d) for 8 patients and standard BU/CY for 5 patients. Two patients received EDX/ATG for SAA.

GVH prophylaxis consisted to; ciclosporine A (CsA, 3 mg/kg/d, iv) plus short methotrexate (MTX, 15 mg on day +1 and 10 mg on days +3 and +6). cyclosporinemia was ranged between 150-400 ng/ml. GI aGVHD was grading as Glucksberg system. All patients were received peripheral blood stem cells (PBSC) grafts. The end point of evaluation was day 100.

Results: Median age was 27 years [17-53]. Median course of chemotherapy pre transplant for malignancies disease was 2 [2 - 5]. HCT-CI score was 0 and 1for 10 and 5 patients respectively. Median dose of CD3+/kg was 4,7x 10^6 [3,9 -6,25]. Median day for neutrophil engraftment was 12 [8-33]. Median day of fever episode was 3[1-10]. Only 2/15 (13%) had infectious diarrhea. NRM at day 100 was 6,6%(1/15 patients).

None of our patients had stage II, III or IV lower GI aGVHD. 6/15 (40%) had stage I GI aGVHD. Interestingly 5/6 patients presented only upper clinical symptoms (vomiting, nausea, anorexia and continued loss weight).

Conclusion: Our preliminary results suggest that allogeneic SCT when without use any antibacterial digestive decontamination prophylaxis was associated with low incidence of severe GI aGVHD without increase risk of digestive infections

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Disclosure of Interest: None declared.

P528

Prospective Longitudinal Study of Late Acute Graft Versus Host Disease after Hematopoietic Cell Transplantation: A Report from Chronic GVHD Consortium

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Introduction: Late acute (LA) graft vs. host disease (GVHD) is persistent, recurrent or new onset acute GVHD symptoms more than 100 days after hematopoietic cell transplantation (HCT). The aim of this analysis is to describe the onset, course, and the outcomes of LA GVHD.

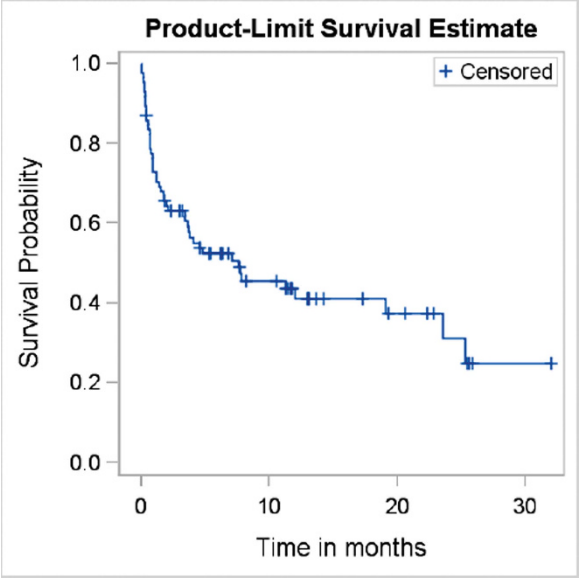
Materials (or patients) and methods: A prospective cohort of patients was enrolled as part of an observational study of immune mediated disorders after HCT within the Chronic GVHD Consortium at 13 centers. Patients already diagnosed with LA or chronic GVHD were excluded.

Results: Out of 913 patients in the study, 85 developed LA GVHD (2-persistent, 40- recurrent and 43 de-novo) for a cumulative incidence of 11% at 2-year after HCT. Median age

was 53.2 years, 70% received URD transplants, and graft source was peripheral blood in 87%. 44% received myeloablative conditioning, and 7% received ATG/ Alemtuzumab. Median time of onset for LA GVHD was 160 days (IQR 128-204) days after HCT with median follow-up for survivors after LA GVHD diagnosis being 10.2 (range 0.7-25.9) months. 60% of patients had biopsy-proven LA GVHD. Single organ involvement at diagnosis was seen in 59 patients (skin 39%, liver 14% and gut 47%), while 26 patients (31%) had $\geq$2 organ involvement. 21% of patients with liver GVHD had only transaminitis without elevated bilirubin and hence were not included for acute GVHD grading but were included in other analyses. Initial treatment included initiation or dose increases of systemic steroids (69%), continuation of calcineurin inhibitors (64%) or topical treatment (52%). Additional immunosuppressive therapy (IST) was added within the first 28 days in 31% or between 28 and 180 days in 24% of patients. Of the alive and evaluable patients, 49/85 (58%) and 48/66 (73%) had a clinical response (CR) at 28 days and 180 days respectively. 36% developed recurrent LA GVHD after a documented CR. The cumulative incidence of chronic GVHD after LA GVHD was 37% (95% CI 27-51%) at one year after LA GVHD diagnosis. 14% discontinued IST without the need to restart it at the time of last follow-up and median duration of IST was 12.8 (range 6.1-24.7) months after HCT. 9% relapsed and 22% died with the main causes of death being GVHD, infection or multi organ failure. Median failure free survival (FFS) as defined by absence of relapse, death or addition of new IST was 7.6 months (95% CI: 3.4-19.1). (Figure) Median overall survival (OS) was 25.3 months. No patient/ transplant or GVHD related factors emerged as significant predictors for OS in univariate analysis and only disease type emerged as significant for FFS (HR for MDS 2.8, $P = 0.009$). In the overall cohort of 913 patients, having classic and/ or LA GVHD was associated with worse OS (HR = 2.5, 95% CI: 1.3-4.6, $P = 0.003$) and higher NRM (HR = 2.5, 95% CI: 1.3-5.09, $P = 0.008$) after HCT, when analyzed as a time-varying covariate.

Conclusion: The overall incidence of LA GVHD is low, but it is associated with prolonged IST and shorter FFS and OS. These results, collected from a prospective longitudinal study, provide valuable information about response and outcomes to serve as a benchmark for conduct of therapeutic trials in LA GVHD.

Disclosure of Interest: None declared.



P529

UGT2B17 minor histocompatibility mismatch and clinical outcome after hla-identical sibling donor stem cell transplantation

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Introduction: Minor histocompatibility antigens (mHags) have been involved in the pathogenesis of graft-versus-host disease (GvHD) and graft-versus-leukemia (GvL) after HLA-hematopoietic stem cell transplantation (HSCT). In this sense, it is well know the higher incidence of GvHD when a male patient receives stem cells from a female donor (thus, involving several mismatches for the antigens codified by the Y chromosome: H-Y). More questionable is the relevance of the autosomal mHags in the clinical outcome after HSCT. UGT2B17 gene deletion may acts as an ubiquitous autosomal mHag and its association with acute GvHD (aGVHD), regardless of the presence of its HLA-restriction molecule (A:29 and/or B:44) has been described.

Materials (or patients) and methods: We carried out a retrospective analysis of UGT2B17 status in a cohort of 1073 patients receiving an HSCT from HLA-identical sibling donors. All were transplanted in Spanish transplant centers between 1996 and 2007. Patients who received a T-depleted graft HSCT were not included. UGT2B17 genotyping was made by polymerase chain reaction sequence-specific amplification (PCR-SSP) with allele-specific primers and method described by Leiden University Medical Center on DNA samples obtained from peripheral blood. Statistical cumulative incidence was estimated for aGVHD, relapse and transplant related mortality (TRM) according to UGT2B17 mismatch. TRM was defined as death due other causes but relapse. The Kaplan Meier method was applied to analyze overall survival (OS) and relapse-free survival (RFS), comparing curves by the log-rank test. Multivariate analysis was performed using the Cox regression model.

Results: UGT2B17 mismatch was present in 6.3% of cases. There were no statistical differences in demographic characteristics between the UGT2B17 mismatched and non-mismatched groups. We did not find statistical differences in cumulative incidence for aGVHD grades II-IV (p: 0.841) but a trend towards signification was seen in univariate analysis when considering only severe aGVHD grades (III-IV; p: 0.095). Multivariate analysis failed to detect a statistical significance. The 5-years-OS was comparable between both groups.

As the presence of the gender mismatch involves many mHags mismatches, thus acting probably in a immunodominant manner, we analysed the effect of an UGT2B17 MM in the absence of H-Y mismatches (803 HSCTpairs). In this population, cumulative incidence of III-IV aGVHDa was significantly higher in the UGT2B17 MM group (25.1% vs 12.9% p: 0.032), and the median OS was 38.8% in MM vs 54.7% non-MM (p: 0.069). There were no statistically significant differences in cumulative incidence of TRM (24% MM vs 25.1% no MM; p: 0.644) or disease relapse (30.6% MM vs 28.7% no MM; p: 0.953).

Conclusion: UGT2B17 mismatch seems to have a clinical impact only when gender mismatch is absent, with an increased incidence of grades III- IV aGVHD. These preliminary results should be confirmed by other studies.

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P530
Grafts Vs Host Disease Prophylaxis By Immunodepletion Of Allogeneic Grafts With Alemtuzumab

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Introduction: T-cell depletion is an effective form for the prevention of GvHD, although the best method for this strategy remains unclear. In an in vitro study we showed 3 log CD3, CD4 and CD8 depletion by incubating 1 mg of alemtuzumab / 1×10^{10} PBPC in the presence of complement with no free antibody in the bag supernatant. In these grafts the reduction of NK cells and other accessory cells was modest.
Materials (or patients) and methods: Now, the outcome of 136 patients with haematological malignancies (acute leukaemia: 74, lymphoma: 35, myelodysplasia: 13, myeloma: 14) transplanted in response between 2006 through 2014 from HLA identical siblings using this strategy was reviewed. The median age was 42 years and 50 patients were older than 45 years. The conditioning was myeloablative (TBI based for lymphoid malignancies, busulfan and melphalan in the remainder) and GvHD prophylaxis was by ex vivo incubation of the PBPC graft with alemtuzumab (''in the bag'' as above: 1 mg / $1x10^{10}$ cells) for 30 minutes followed by cyclosporine until day +90. Donors were unrelated to 16 patients and in addition they received ATG (rabbit, 5 mg.kg x 3 days).
Results: The median CD34+ harvested count was 6.25 x 10^6/kg and the median alemtuzumab dose in PBPC grafts was 5.5 mg. 132 patients engrafted at median of 12 days. Graft vs. host disease (=/> grade 2) was seen in 16% and was associated with bacterial infections. Reactivation of CMV was documented in 20% who were all successfully treated with gancyclovir. One year non relapse mortality was 17%, while malignancy recurred in 20%. In 3 patients TRM was post-transplant lymphoma, lung carcinoma and cardiomyopathy. At a median follow up of 1000 days, overall survival is 68%. Cox analysis showed that mortality was significantly related to GvHD ($P = 0.002$). There was no difference in outcome following the 2 conditioning strategies, or according to the source of the grafts.
Conclusion: We conclude that ex vivo dose adjusted alemtuzumab based on cell content of the PBPC graft led to low incidence of GvHD, low CMV reactivation rate and satisfactory overall survival. Myeloablative conditioning may be a necessary component for certain malignancies and in the absence of sever forms of GvHD may lead to improved outcomes of allogeneic transplants.
Disclosure of Interest: None declared.

P531
Toll-like receptor 2,4,7 and 9 expression, signaling and tolerance in patients with acute and chronic graft-versus-host disease

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Introduction: Graft vs Host Disease, characterized by damage of epithelial surfaces in target organs caused by donor-derived T-cells, is the leading cause of non-relapse mortality and morbidity post allogeneic stem cell transplantation (allo-HSCT). Costimulatory signals delivered by APCs, a process mainly mediated through Toll-like receptors (TLRs), is also associated with the onset of GvHD.
Materials (or patients) and methods: Given the controversy governing the role of TLRs in GvHD development, we studied TLR2,4,7,9 expression by qRT-PCR and flow cytometry, in peripheral blood mononuclear cells (PBMCs), purified lymphocyte subsets and target tissues from acute (aGvHD, $n = 35$) and chronic (cGvHD, $n = 49$) GvHD patients as compared to no-GvHD patients ($n = 48$). In addition, the PBMCs response to specific TLR2,4,7 and 9 agonist stimulation (HKLM, LPS, R848, CpG-ODN respectively) was investigated among the different patient groups and the TLR signaling profile, both in unstimulated and agonist-stimulated PBMCs, was assessed by PCR-array analysis.
Results: An up-regulation of TLR2,4 expression in aGvHD PBMCs was observed, both at transcript ($P < 0.05$, $P < 0.001$) and protein level ($P < 0.05$, $P < 0.001$), whereas cGvHD PBMCs overexpressed TLR2,4 mainly at protein level ($P < 0.05$, $P < 0.001$). Immunomagnetically selected cells displayed an up-regulation of TLR4 protein expression in aGvHD T-($P < 0.05$) and B-cells, while both lymphocyte subsets tended towards significant TLR4 overexpression at transcript level. Immunohistochemistry in skin biopsies from both cGvHD and aGvHD groups showed a strong up-regulation inTLR2 and 4.Up-regulated intracellular TLR7,9 expression in salivary glands of cGvHD patients was observed, while in contrast, all TLRs were down-regulated in oral mucosa sections. Distinctive TLR expression was also detected in large intestine biopsies. The TLR signaling pathway in PBMCs confirmed the qRT-PCR data as regards TLR2,4 up-regulation in aGvHD over no-GvHD samples, while the gene expression pattern of acute and chronic GvHD patients was similar; the transcription factor FOS and the cytokines CSF3 and IL8 were up-regulated, while the CD180, IL-2 and TLR-10 were down-regulated in both groups, albeit without or with borderline, statistical significance. Interestingly, post TLR ligand activation, the mean fold change of TNF-α, IFN-β and IL-6 measured by qRT-PCR in PBMCs of both aGvHD and cGvHD patients was significantly lower as compared to control group, suggesting marked hyporesponsiveness to TLR stimulation when GvHD is established. Similar findings were detected by ELISA quantitation of pre/post TLR activation ratio of TNF-α from GvHD and no-GvHD samples. PCR-array studies in TLR ligand-stimulated PBMCs confirmed the hyporesponsiveness in the entire TLR signaling pathway during GvHD, most prominently in the aGvHD-derived samples. Moreover, distinct gene expression patterns were observed depending on the extracellular (TLR2,4) or intracellular (TLR7,9) stimulation of aGvHD and cGvHD PBMCs.
Conclusion: Our data support the involvement of TLR signaling pathway in GvHD and describe for first time, the TLR2,4,7,9 expression and signaling pathway as well as a TLR tolerance status of PBMCs in established aGvHD and cGvHD. It is implied that the induction of TLR tolerance to PBMCs, by repeated exposure to TLR agonists before alloHSCT, may serve as a novel anti-GvHD prophylaxis.
Disclosure of Interest: None declared.

P532
Serum MicroRNAs MiR-146a, MiR-199, MiR-93* and MiR-423 as Biomarkers for Graft Versus Host Disease

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Introduction: Despite graft-versus-host disease (GvHD) being a major complication of allogeneic hematopoietic stem cell transplantation (HSCT), no markers are routinely used in the clinic to aid early detection or severity monitoring. MicroRNAs are small RNAs that repress translation. They are present in body fluids, including within exosomes, and have been associated with GvHD and immune function.

Materials (or patients) and methods: 6 microRNAs (miR-146a, miR-155, miR-423, miR-199a-3p, miR-93* & miR-377) were investigated in total serum ($n = 34$) and serum exosomes ($n = 15$) samples from HSCT patients every 7 days pre to post-HSCT (day-7 (D-7) to D28) by TaqMan® qRT-CPR.
Results: The patient cohort (25(74%) male, 9(26%) female) comprised of 7(21%) sibling (SIB) and 27(79%) matched unrelated donor (MUD) allogeneic peripheral blood stem cell (PBSC) transplants. Nineteen (56%) patients developed acute GvHD (aGvHD) (6 = grI, 8 = grII, 5 = grIII) and 16(47%) chronic GvHD (cGvHD) (6 = limited, 10 = extensive, 3 = status unknown).
Acute GvHD patients had significantly higher expression of miR-146a and miR-93* at D14 ($P = 0.02$ & $P = 0.02$) and miR-423 at D0/D7/D14 & D28 ($P = 0.04$, $P = 0.03$, $P = 0.03$ & $P = 0.03$) compared to no GvHD patients. The same microRNAs were also associated with aGvHD incidence at D14 by ROC analysis (miR-146a $P = 0.02$, AUC = 0.82; miR-93* $P = 0.05$, AUC = 0.76; miR-423 $P = 0.02$, AUC = 0.80). No microRNA was differentially expressed between pre-GvHD and time of aGvHD onset ($P > 0.05$), nor associated with incidence of cGvHD at any time point ($P > 0.05$).
Assessing aGvHD severity, miR-199 expression was significantly higher in severe (III) vs. mild (I-II) GvHD ($P = 0.02$) and vs. no GvHD ($P = 0.008$) at D14. Expression of miR-146a and miR-93* at D14 significantly differentiated severe (III) from no GvHD ($P = 0.03$ & $P = 0.004$), but not from mild (I-II) GvHD ($P > 0.05$). No microRNAs were associated with patient gender or donor relation (SIB/MUD) ($P > 0.05$). MiR-146a ($P = 0.005$), miR-199 ($P = 0.006$), miR-423 ($P = 0.031$) and miR-377 ($P = 0.024$) were expressed at significantly higher levels in patients who did not receive RIC at the time of HSCT (D0).
A subset of patients ($n = 15$; 5 = no GvHD, 4 = grI, 1 = grII, 3 = grIII) were assessed for microRNA expression within serum exosomes. MiR-155, miR-146a and miR-93* expression was lower in aGvHD vs. no GvHD at D14 ($P = 0.06$, $P = 0.06$ & $P = 0.01$). MiR-377 demonstrated higher expression in aGvHD vs. no GvHD at D0 ($P = 0.04$) and D7 ($P = 0.03$). Exosome microRNAs were not associated with aGvHD severity, nor incidence of cGvHD ($P > 0.05$).
MiR-155 (D28 $P = 0.004$), miR-146a (D7 $P = 0.04$, D14 $P = 0.006$ & D28 $P = 0.02$), miR-199 (D0 $P = 0.03$ & D7 $P < 0.001$), and miR-93* (D-7 $P = 0.04$, D0 $P = 0.02$, D14 $P = 0.01$ & D28 $P = 0.01$) were expressed at significantly higher levels in exosomes compared to total serum. MiR-377 expression was higher in total serum compared to exosomes at D-7 ($P = 0.006$) and D28 ($P = 0.01$).
Conclusion: This investigation demonstrates the potential of serum miR-146a, miR-93*, miR-423 and miR-199 as biomarkers for GvHD, assessed using robust and non-invasive methodology. Several microRNAs were expressed at a significantly higher level in serum exosomes compared to total serum, suggesting potential biologically activity during HSCT. Identification of the function and targets of these microRNAs, as well as their specific role within exosomes, may advance understanding of GvHD pathobiology, as well as aid in early detection and prophylactic treatment.
Disclosure of Interest: None declared.

P533
Urinary MicroRNA Expression is Associated with Incidence and Severity of Graft Versus Host Disease

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Introduction: Recently, microRNAs have been implicated as useful biomarkers to predict incidence and severity of graft versus host disease (GvHD), a serious complication of haematopoietic stem cell transplantation (HSCT). MicroRNAs are small RNAs that regulate ~50% of genes by repressing translation and have been identified in exosome, where they are protected from degradation.
Materials (or patients) and methods: Expression of 6 microRNAs (miR-146a, miR-155, miR-423, miR-199a-3p, miR-93* & miR-377) was assessed in whole urine and urine exosome samples taken from HSCT patients ($n = 30$) every 7 days pre to post-HSCT (day -7 (D-7) to D28), as well as healthy controls, by TaqMan® qRT-CPR. Expression of hemoxygenase-1 (*HMOX1*) and transforming growth factor beta (*TGFB1)*, putative microRNA targets or regulators, was also assessed by TaqMan® qRT-PCR.
Results: The 30 patient cohort (19(63%) male, 11(37%) female) comprised of 9(30%) sibling (SIB) and 21(70%) matched unrelated donor (MUD) allogeneic peripheral blood stem cell (PBSC) transplants. Twenty (67%) patients developed acute GvHD (aGvHD) (9 = grI, 7 = grII, 4 = grIII) and 12 (48%) developed chronic GvHD (cGvHD) (9 = limited, 3 = extensive, 5 = status unknown).
Significantly higher expression of miR-377 was observed in aGvHD vs. no GvHD at Day 0 (D0) ($P = 0.02$) and D14 ($P = 0.04$) and further demonstrated by ROC analysis (D0 $P = 0.03$, AUC 0.78; D14 $P = 0.05$, AUC 0.74). MiR-377 expression was also higher in classic aGvHD (aGvHD < 100 days post-HSCT) vs. no GvHD ($P = 0.04$; ROC $P = 0.03$, AUC = 0.71). MiR-199 was expressed at a lower level in cGvHD compared to no cGvHD and this was significant at D-7 ($P = 0.01$), D14 ($P = 0.04$) and D28 ($P = 0.04$).
Regarding aGvHD severity, miR-423 expression was significantly lower in severe (III) vs. mild (I-II) aGvHD at D0 ($P = 0.004$; ROC $P = 0.01$, AUC = 1.0), D7 ($P < 0.001$; ROC $P = 0.005$, AUC = 0.98) & D28 ($P = 0.047$; ROC $P = 0.05$, AUC = 0.95) and similarly, in severe vs. no aGvHD (D0 $P = 0.014$, D7 $P < 0.001$ and D28 $P = 0.05$). MiR-93* expression was significantly lower in severe vs. mild aGvHD at D0 ($P = 0.047$; ROC $P = 0.03$, AUC = 0.93).
Pre-HSCT expression of miR-146a, miR-199 and miR-377 was significantly higher in aGvHD vs. healthy controls ($P < 0.001$, $P = 0.010$, $P < 0.001$, respectively) and HSCT patients vs. controls ($P < 0.001$, $P = 0.007$ and $P < 0.001$, respectively).
MiR-155, miR-146a, miR-199 & miR-377 (D-7 & D0) were expressed at significantly higher levels in urine exosomes compared to whole urine. However, no microRNA was predictive for aGvHD or cGvHD in urinary exosomes ($P > 0.05$). The mRNA expression of HO-1 (*HMOX1*) and TGF-β (*TGFB1*) was assessed in urinary total RNA at D0 and D14. *HMOX1* expression at D0 was significantly lower in cGvHD vs. no GvHD ($P < 0.01$). *TGFB1* was not significantly associated with aGvHD or cGvHD ($P > 0.05$). With regard to microRNAs, there was a significant inverse correlation between miR-146a vs. *TGFB1* at D14 ($P < 0.001$) and between miR-155 vs. *TGFB1* at D0 ($P < 0.001$).
Conclusion: This study shows the potential of urinary miR-377, miR-423, miR-199 and miR-93* as biomarkers for GvHD, assessed using robust and non-invasive methods. Results are being validated in an expanded independent cohort. Further studies into the function and targets of these microRNAs may advance understanding of GvHD pathobiology as well as aid in early prophylactics and severity monitoring. Analysis of exosomal microRNA expression in relation to HSCT is warranted.
Disclosure of Interest: None declared.

P534
IRAK1 and miR-146a for predicting the outcome of allogeneic haematopoietic stem cell transplantation

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Introduction: Micro-RNAs (miRNAs) have been shown to be potent regulators of various biological processes. MiR-146a

controls the innate immune cell and T-cell responses, and its deficiency causes autoimmunity. The interleukin-1 receptor-associated kinase (IRAK1) is an established target gene of miR-146a. IRAK1 regulates multiple pathways in both the innate and adaptive immune system by linking several immune-receptor-complexes to TNF receptor-associated factor. In this study we investigated the association of two SNPs in miR146a (rs2910164, G->C transversion and rs2431697, T->C transition) and one SNP in the IRAK1 gene (Rs3027898, A->C transition polymorphism in the 3'-UTR) on the outcome of allo-HSCT.

Materials (or patients) and methods: The study was performed on $n = 1384$ peripheral blood samples (patient-donor pairs) collected after allo-HSCT. Genotyping was performed by KASP, based on competitive allele specific PCR (LGC Genomics, UK), to assess rs3027898 = IRAK1, and the 2 variables of miR-146a; rs2910164 = miR-146a(1) and rs2431697 = miR-146a(2). Statistical analyses were performed using SPSS v22.0 and R v3.1.2.

Results: Rs3027898 = IRAK1 analysis showed that the 'C' allele promoted the incidence of relapse ($P = 0.014$) (Figure1) and also associated with an increase in non-relapse mortality ($P = 0.020$). This allele was also associated with a reduced overall survival in patients who underwent non-T cell depleted allo-HSCT ($P = 0.022$). In patients, no significant association with HSCT outcome was observed in the T cell depleted and non-T cell depleted cohorts. In donors, the G allele in rs2431697 of miR146a was significantly associated with a better overall survival ($P = 0.024$).

Conclusion: In study we highlighted the effect of IRAK1 genotype on the outcome of allo-HSCT. The C allele in IRAK1 allowed a higher risk of relapse and had negative effect on the non-relapse mortality. IRAK1 is a validated target of miR-146a, which is known to promote expression of interferon genes via the NF-kB pathway. The 'G' allele in rs2431697 of miR146a allowed a better overall survival this may be explained by the fact that miR-146a functions as a negative regulator of donor T cells in GvHD by targeting TRAF6, leading to reduced TNF transcription. Many SNPs, along with other polymorphisms, regulate the function of immune cells, their receptors, effector molecules, cytokines and chemokines. The assessment of these variants in the pre-transplantation setting may improve risk assessment and prediction of HSCT outcome on an individual patient base.

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P535

Quantification of metabolically active by tetrazolium salt: a rapid assay for the validation of extracorporeal photopheresis procedure

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Introduction: For the treatment of acute and chronic steroid-refractory graft versus host disease (GvHD) extracorporeal photopheresis (EP) represents a second-line therapy. Its clinical effect is based on the immunomodulatory action exerted on mononucleated (MNC) cells of patient by ultraviolet A light (UVA)-activated 8-methoxypsoralen (8-MOP), making a bond between DNA nucleic bases resulting in cell apoptosis and death. For the therapeutic use of human cells, Regulatory Agency prescribes the validation of procedures, often performed, in the case of EP, by evaluating cell proliferation or cell apoptosis by flow cytometry. Here, we have validated the EP procedure by tetrazolium salt quantification (WST-1) of metabolically active cells: the number of metabolically active cells before and after treatment has been correlated with the amount of formazan derived by the cleavage of WST-1 by mitochondrial dehydrogenases. Samples were tested in parallel evaluating cell proliferation by Carboxyfluorescein succinimidyl ester (CFSE) and cell apoptosis and death by 7-aminoactinomycin D (7AAD)-Annexin V staining.

Materials (or patients) and methods: EP, performed on patients with GvHD ($n = 6$) using the off-line Vilbert-Lourmat method, consisted in the collection of MNC using a COBE SPECTRA apheresis system, followed by irradiation of MNC with UVA light for 10 minutes adjusted to 2 J/cm2 (Macogenic, Macopharma, France) in presence of 200 ng/ml of 8-MOP. For WST1 analysis, before and after irradiation, 100.000 cells were plated in 96 well plate in quadruplicate. Immediately after treatment and after 2 and 4 days of culture,10 μl of WST-1 was added in each well; the absorbance of formazan was measured by ELISA after 4 hours of incubation. Results were expressed as: 100X absorbance of sample post EP/absorbance before EP. In the CFSE assay, 200.000 cells, resuspended in RPMI,10% FBS,1% L-glutamine, were labeled for 10 minutes at 37 °C, 5% CO_2 with CFSE (5μM) before and after irradiation. After 4 days of culture in the presence of IL2 (500 U/ml) and anti-CD3 (500 ng/ml), CFSE staining on CD45(+) gated cells was analyzed by flow cytometry. The inhibition of cell proliferation induced by the treatment was calculated as follows:100X(% CFSE pre

Results: After four days from irradiation, the residual enzymatic activity was only $34.5 \pm 15.5\%$ ($n = 4$); this data correlates with the increase of apoptosis ($19.4 \pm 14.1\%$ respect to pre EP at day 4, $P < 0.05$, $n = 4$) and with the inhibition of proliferation confirmed by CFSE ($94.7 \pm 2.5\%$. $n = 6$).

Conclusion: WST-1 assay could represent a rapid and easy test for the routinely quality control of EP treatment.

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P536

The impact of referral >12months from diagnosis of cGVHD on response to ECP therapy: A Single Photopheresis Centre Experience

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Introduction: Chronic GVHD (cGVHD) is a major cause of morbidity and mortality post allogeneic stem cell transplantation. Extracorporeal Photopheresis (ECP) is one of the recommended therapeutic options for Steroid Refractory (SR) cGVHD. Although guidelines suggest early initiation of second-line therapy, accessibility to an ECP centre may be a limiting factor. In recent years increasing numbers of allogeneic transplant centres are gaining access to ECP services. It is not clear whether patients who have had longstanding SR cGVHD will gain any benefit from late intiatiation of ECP therapy.

Materials (or patients) and methods: A search of our registry database identified 191 patients that were referred for ECP, from multiple transplant centres, for treatment of cGVHD between 1996-2014. A total of 102 patients (94 adult, 8 paediatric) were included. 88 patients were excluded due to incomplete data regarding time of cGVHD diagnosis. Response was defined as ≥50% reduction in steroid dose. All patients were started on a treatment schedule of 1 cycle (= 2 ECP treatments on consecutive days) fortnightly for 12 weeks after which the interval between treatments was tailored according to response.

Results: The outcome measures analysed in groups according to time of referral are summarized in the table below.

Time from cGVHD diagnosis to ECP referral	*Number of patients*	*Mean steroid dose at start of ECP therapy (mg)*	*Number of Responders at cycle 28*	*Number of Responders at cycle 56*	*Number who had stopped steroids by cycle 56*	*Median Duration of ECP (weeks)*
0-3 months	10	52.8	10 (100%)	10 (100%)	6 (60%)	96.4
>3-6 months	23	26.0	16 (70%)	21 (91%)	11 (48%)	100.1
>6-12 months	26	20.9	19 (73%)	24 (92%)	15 (58%)	66.9
>12months	43	16	29 (67%)	22 (51%)	20 (47%)	107.4
	Total = 102		$P = 0.216$	$P = 0.017$	$P = 0.746$	$P = 0.543$

The mean time to ECP referral from diagnosis of cGVHD was 17.2 months. The dose of steroids at ECP initiation was higher in groups that were referred for ECP earlier. As expected, overall there was a trend for increasing response rate with earlier referral. The association between time to referral and response rate was statistically significant ($P = 0.017$, Fisher's test) at the cycle 56 assessment timepoint but not at the cycle 28 assessment (Chi squared). The overall median duration of ECP was 93.4 months(Kaplan-Meier plot), the difference in ECP duration between the four groups was not statistically significant. Quality of Life (QoL) data available in 8/43 patients referred >12months from cGVHD diagnosis suggested a trend towards an improvement; mean score 36.6 (Lee cGVHD symptom scale) at the start of ECP reducing to a mean score of 18.2 at cycle 56.

Conclusion: When considering ECP as a treatment for SR cGVHD, early referral is optimal and yields greater response to therapy. However, our results show that 51% of patients referred >12months after diagnosis of cGVHD responded to ECP and 47% were able to stop steroids. The data suggests that patients with a longstanding history of cGVHD may still benefit from late intervention with ECP. This finding is of particular relevance to centres, that have acquired access to ECP, with cohorts of patients with SR cGVHD. The results need to be validated in prospective studies using NIH response criteria and Quality of Life assessments.

Disclosure of Interest: None declared.

P537

Differential microRNA expressions in cutaneous acute graft-versus-host disease

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Introduction: Graft-versus-host disease (GVHD) is still one of the major complications of allogeneic stem cell transplantation (allo-HSCT). Classically, GVHD has been divided into acute (aGVHD) and chronic. MicroRNAs (miRNAs) have reported regulatory roles in both health and disease. Recently, their functions in GVHD have also been highlighted in serum, plasma and gut biopsies. The aim of this investigation was to identify microRNAs specific to the frequently affected aGVHD target organ, skin as well as to test whether miRNA expression levels were associated with overall survival.

Materials (or patients) and methods: The Exiqon global microRNA profiling (739 miRNAs) by RT-qPCR was performed on a discovery cohort that identified a signature microRNA list in skin biopsies obtained from patients ($n = 5$) at the time of cutaneous histopathological aGVHD onset (grades I-III) and healthy volunteers ($n = 4$). MicroRNA expression in the validation cohort was performed by individual RT-qPCRs and consisted of skin biopsies from pre-transplantation ($n = 6$), post-transplantation ($n = 17$) and healthy volunteers ($n = 6$). MiR-34a-5p protein targets p53 and c-Myc were then evaluated via immunohistochemistry in the same cohort and the positivity score calculated using the quickscore method. The discovery and validation cohort consisted of classical aGVHD patients (onset date ≤ 100 days post allo-HSCT) and skin histopathological grades were used for all the analyses. The patients in the validation cohort were grouped into 0-I (none-mild) and II-III (moderate-severe) skin histopathological aGVHD grade.

Results: Distinct microRNA expression clusters existed between post allo-HSCT and normal skin biopsies. Twelve miRNAs (miR-142-3p, miR-34a-5p, miR-34a-3p, miR-503-5p, let-7c-5p, miR-21-3p, miR-365a-3p, miR-23b-3p, miR-493-3p, miR-200b-3p, miR-24-3p and miR-2110) were selected for further validation. Overall Kruskal-Wallis analysis-of-variance showed that miR-34a-5p ($P = 0.0005$), miR-34a-3p ($P = 0.013$), miR-503-5p ($P = 0.021$) and let-7c-5p ($P = 0.037$) were elevated and significantly involved in allo-HSCT procedure (GVHD and overall survival). There was a significant interaction ($P = 0.016$) for miR-34a-3p and miR-503-5p which was diagnostic of aGVHD. Likewise, both miRNAs were significantly (miR-503, $P = 0.04$; miR-34a-3p, $P = 0.03$) associated with overall survival independent of clinical risk factors. MiR-34a-5p expression and p53-positive cells were significantly positively correlated in the epidermis of allo-HSCT patients.

Conclusion: This investigation is the first to report miRNA investigation in the GVHD target organ, the skin. MiR-503-5p targets the CD40 gene and is also involved in the differentiation of monocytes to macrophages. CD40-CD40 ligand is important in T cell activation and has been extensively studied in GVHD. Results suggest that during GVHD, miR-503-5p may be overexpressed to regulate T cell activation as well as to direct more monocyte to macrophage differentiation. MiR-34a-5p expression has been shown to be up-regulated in aGVHD gut biopsies which supports the similar overexpression pattern observed in the skin. The positive correlation between miR-34a-5p and p53-positive cells may be associated to the apoptosis observed in GVHD skin. Therefore, this study has shown that microRNA expression levels in clinical skin biopsies obtained at the time of aGVHD onset could potentially be used as predictive biomarkers for overall survival.

Disclosure of Interest: None declared.

P538

MiR-146a and miR-155 expression levels in allogeneic haematopoietic stem cell transplantation patients is predictive of acute graft-versus-host incidence

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Introduction: Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for numerous haematological malignancies. Graft-versus-host disease (GVHD) is a major cause of mortality and is classified into acute (aGVHD) and chronic. MicroRNAs (miRNAs) play a significant role in inflammatory diseases and have reported potentials as biomarkers of different diseases. MiR-146a and miR-155 have established immune-related functions in both the innate and the adaptive immune system. The aim of this investigation was to test whether the expression levels of miR-146a and miR-155 quantified in whole blood could be used to predict aGVHD-onset prior to disease manifestation. The targets of miR-146a (TRAF6, IRAK1, STAT1-α and IRF5) and miR-155 (STAT1-α) were also studied to understand the regulatory mechanism. Since *SPI1* (*PU.1*) is an important primary myeloid transcription factor its gene expression levels were also tested in relation to both miRNAs.

Materials (or patients) and methods: Whole blood samples were collected in PAXgene™ Blood RNA tubes from pre-transplant (Day-7, $n = 35$) and post-transplant (Day + 28,

$n = 54$) patients. The PAXgene Blood miRNA kit was used to extract total RNA from whole blood. Individual Taqman (Life Technologies, USA) miRNA (miR-146a, miR-155 and SNORD48) and gene expression (TRAF6, IRAK1, STAT1-α, IRF5, SPI1 and GAPDH) assays were used to synthesize cDNA and quantify the specific miRNA and gene expression levels. SNORD48 (001006) was used as the reference control for the miRNA studies and GAPDH for the gene expression investigations. Sera were used to test the protein levels of the targets (TRAF6, IRAK1, STAT1-α and IRF5), using commercially available Enzyme-Linked Immunosorbent assays (CUSABIO, China). Binary logistic generalised linear models were used to test for the main and interaction effect of both miRNAs and Spearman correlation to test for relationship between the miRNAs and the target expression levels (SPSS v21).

Results: Results showed that miR-146a and its interaction with miR-155 was significantly ($P = 0.025$) predictive of aGVHD incidence in pre-disease onset (Day + 28) samples. For every unit increase in the expression of both miR-146a and miR-155 the risk of aGVHD incidence declined 0.71 times. However, none of the targets correlated with the miRNA expression levels. Interestingly, the expression levels of miR-146a (rs = -0.45, $P = 0.016$) and miR-155 (rs = -0.534, $P = 0.004$) negatively correlated with *SPI1* (*PU.1*).

Conclusion: Statistical interactions are often overlooked in transplantation studies. This investigation has shown that lower expression levels of both miR-146a and miR-155 in patients prior to aGVHD onset can be predictive of aGVHD incidence. This finding is supported by the recent mouse investigation where in lower miR-146a expression levels were indicative of severe aGVHD. The 'check-point' differential expression mechanism suggested in the literature also supports the finding that both miRNAs are involved in regulating inflammation. The negative correlation between both miRNAs and SPI1 may suggest that during the graft-versus-host reaction, the miRNAs regulate the expression of this master transcription factor instead of being activated by it. Overall, this study has shown that the interaction of miRNAs with outcome may be used to predict aGVHD incidence.

Disclosure of Interest: None declared.

P539

IL-17 decreases during acute Gastro-Intestinal GvHD and is produced by non CD4 T cells

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Introduction: Previous studies suggested that interleukin 17 (IL-17) produced by TH17 cells worsens Graft versus Host Disease (GvHD) in mouse model and in human hematopoietic cell transplant recipients. Since the gastrointestinal tract represents one of the most severely damaged organs during acute GvHD, we aimed to analyse the cellular production of IL-17 in GI tract *in situ*.

Materials (or patients) and methods: We analysed 159 biopsies obtained at different timepoints after allogeneic transplantation. All patients gave informed consent to prospective sampling of biopsies and data analysis. Immunohistology was performed by single antibody staining for IL17 and double immunofluorescence for IL17/CD3 and IL17/CD4. RORyt PCR was performed on RNA obtained from 169 biopsies.

Results: Immunohistochemistry by single antibody staining of IL-17 positive cells surprisingly revelaed downregulation of IL-17 positive cells in more severe GvHD (Mean +/- SEM for GvHD stage 0 = 3.07 +/- 0.53; GvHD stage 1 = 2.11 +/-0.39; GvHD stage 2-4 = 1.55 +/-1.10; $P = 0.01$) We further analysed transcription factor for IL-17, RORyt, at mRNA level in 169 biopsies and found GvHD dependent downregulation of RORyt gene (Mean +/- SEM for GvHD stage 0 = 5.16 +/- 0.44; GvHD stage 1 = 3.58 +/-0.0.36; GvHD stage 2-4 = 2.67 +/-0.27; $P = 0.001$) which supports our cellular results. In order to define the cellular source of IL17 production we next established double staining for immunofluorescence of CD3 + IL-17 and CD4 + IL-17. Analyses on 39 patient biopsies so far revealed that IL-17 producing cells are negative for CD3 and CD4 cells.

Conclusion: In conclusion, we observed downregulation of IL17 both on the level of IL17 producing cells and IL17 transcription factor RoRyt in human GvHD. Double staining indicated that this effect is not mediated by TH17 cells but by IL17 producing non T cells. As these cells are downregulated in more severe GvHD they might be protective NKT or innate lymphoid cells. Further studies are planned to characterize these cells and their protective factors.

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P540

Day 28 Response is an Important Timepoint for Measurement of Efficacy after Second Line Treatment with Extracorporeal Photopheresis (ECP) for Acute Graft-versus-Host Disease

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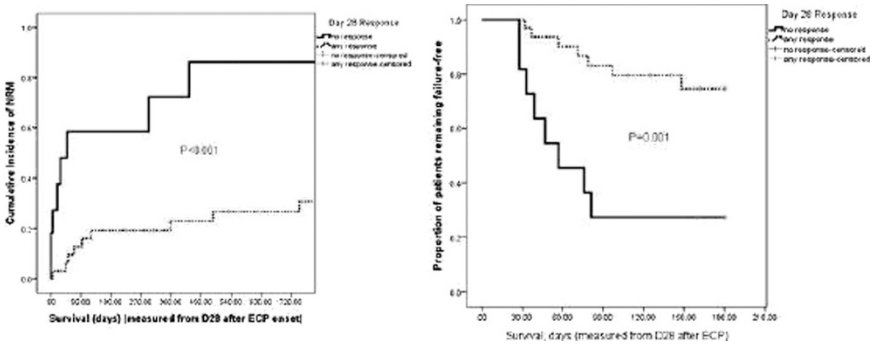
Introduction: Day 28 (D28) response after steroid therapy for grade (gr.) 2-4 acute GVHD (aGVHD) has been correlated with overall survival (OS) and non-relapse mortality (NRM), and is considered an early surrogate measure for clinical trials. Recently, a novel composite endpoint of GVHD-free, relapse-free (GFRS) survival [no grade 3-4 aGVHD, chronic GVHD (cGVHD) requiring systemic therapy, or relapse], at 1 y has been proposed to be a better measure of hematopoietic cell transplant (HCT) success. We have previously shown that extracorporeal photopheresis (ECP) is effective as 2nd line therapy and is associated with 6 month failure free survival (6 mFFS) with failure defined as death, treatment change or cGVHD. We hypothesized that D28 response after ECP initiated for 2nd line therapy is a useful early surrogate for survival, including 6mFFS and 1y GRFS.

Materials (or patients) and methods: Adults patients (pts) treated with ECP as 2nd line therapy for gr. 2-4 aGVHD after their first HCT for hematologic malignancies from 2007-2013 were included. Response to ECP at D28 was assessed and analyzed as previously described in an ASMBT joint statement. We studied the association of D28 response with 2 y OS, NRM, and 1 y GRFS, all calculated from D28 after start of ECP.

Results: Forty-three pts with a median age of 49 y (range 20-67) who underwent HCT were included. The majority (74%) underwent unrelated HCT with 47% ablative and 53% reduced intensity conditioning with 65% PBSC as graft source from matched donors (74%) or mismatched (26%) donors as treatment for lymphoid/plasma cell disorders (26%), acute leukemias/MDS (65%) or CML/MPN (9%). GVHD prophylaxis was either calcineurin inhibitor + methotrexate (34%) or MMF (66%) based. ECP was initiated as 2nd line therapy for gr. 2 (26%), 3 (44%), or 4 (30%) aGVHD with median time to ECP of 24 d. Steroids were escalated in 88% of pts prior to ECP, and 49% of pts were steroid refractory (SR). Gr. 3-4 aGVHD was seen in 63% of pts who were SR compared to 9% of pts with gr. 2 aGVHD ($P = 0.004$). D28 response after ECP was partial (33%), very good partial (12%), complete (30%) and no response (25%) with overall response rate of 74%. Two y OS was 47.4% (standard error [SE] ± 0.078) and NRM was 43.6% (SE ± 0.081). Gr. 3-4 aGVHD ($P = 0.025$) and SR aGVHD ($P = 0.007$) were associated with increased NRM. D28 responders had a 2 y NRM of 31% compared to 86% for non-responders ($P < 0.001$) (Figure 1). In multivariable analyses (MV), adjusted for aGVHD gr. (2 vs. 3-4), steroid response prior

[P540]



to ECP (SR vs. non SR), D28 ECP response remained an independent predictor of decreased NRM (HR 0.26, 95% CI 0.096-0.7, $P = 0.009$). Six month FFS was higher in D28 responders (79.5%) compared with non-responders (27.3%) ($P = 0.001$)(Figure2). In MV adjusted for aGVHD gr. (2 vs. 3-4) and steroid response prior to ECP (SR vs. non SR), D28 response to ECP predicted for a lower NRM (HR 0.30, 95% CI 0.1-0.93, $P = 0.035$). One y GRFS was 25.6% (SE $\pm$0.067). D28 ECP response did not influence 1 y GRFS.

Conclusion: D28 is a reliable time point for assessment of efficacy of ECP as 2nd line therapy for aGVHD and is associated with 6m FFS and 2 y OS. D28 non-responders have high NRM, and should be considered for further intervention. D28 response assessment should be incorporated in future clinical trials involving ECP for 2nd line therapy for aGVHD.

Disclosure of Interest: None declared.

P541

Association of the serum concentration of cyclosporine with the occurrence of moderate to severe chronic graft versus host disease in allogeneic hematopoietic stem cell transplantation from a matched sibling donor

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Introduction: Cyclosporine A (CsA) is a widely used drug for prevention of graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, target blood levels varies among institutions and it has been rarely been studied that how the blood level of CsA affect the incidence of chronic GVHD.

Materials (or patients) and methods: A total of 183 patients who underwent allo-HSCT from an HLA-matched sibling donor between Sep 2006 and Jun 2014 were retrospectively reviewed. Peripheral blood was used as a stem cell source in all cases, and the patients received methotrexate and CsA as a prophylaxis regimen for GVHD.

Results: The median age at allo-HSCT was 45 years. Acute leukemia was most common ($n = 124$, 67.8%), and myelodysplastic syndrome ($n = 24$ 13.1%), and aplastic anemia ($n = 11$, 6.0%) came next. The average CsA serum concentration (CsA^{avr},ng/ml) during a post-transplant period (0-1 month, 1-2 month, and 2-3 month after allo-HSCT) was calculated in each patient. The median value of CsA^{avr} was 223.0, 194.8, and 157.2 during a period of 0-1, 1-2 and 2-3 month. When grouping patients by CsA^{avr} level (group 1: <200; group 2: ≥ 200 and <250; group 3:≥ 250), the incidence of acute GVHD$\geq$Gr.2 was significantly frequent in group 1 during 1-2 month (17.6% vs 2.2% vs 3.6%, $P = 0.011$). In Cox-proportional hazard model, CsA^{avr} between 0 to 1 month showed significant association with the occurrence of moderate to severe cGVHD in univariate and multivariate analysis; the incidence of cGVHD was lowest in group 2 ($P = 0.017$ in univariate analysis; $P = 0.002$ in multivariate anaylsis adjusted for gender, age, underlying disease, reduced intensity conditioning, total body irradiation, anti-thymocyte globulin, CsA^{avr} during 0-1, 1-2, and 2-3 month). Disease free survival did not differ according to CsA^{avr} during 0-1, 1-2, and 2-3 month after allo-HSCT.

Conclusion: Blood level of CsA between 200 and 250 mg/ml during the first month after transplantation was significantly associated with the less occurrence of moderate to severe cGVHD.

Disclosure of Interest: None declared.

P542

Analysis of Immunological changes occurring in the peripheral blood of patients treated with extra corporal photopheresis (ECP) for chronic graft versus host disease (cGvHD)

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Introduction: Chronic graft-versus-host disease (cGVHD) develops in more than 50% of survivors of allogeneic stem cell transplantation and is responsible for mortality in one third of patients[2]. Long term immunosuppressive therapy with steroids is the standard treatment. Extracorporeal photopheresis (ECP) is successful in about 50% of patients after 3–6 months of therapy. The exact mechanism of action of ECP is not well understood. In this prospective IRB approved study, immune parameters were analyzed in the leukophoresed blood of patients with cGvHD undergoing ECP.

Materials (or patients) and methods: Allogeneic HSCT recipients who had steroid dependent/resistant extensive cGVHD or were steroid intolerant were eligible to participate. Patients who had received Rituximab in the past three months were excluded. We studied 22 patients with cGVHD undergoing ECP. All subjects underwent baseline, two, four and six-month assessments. 10 ml of leukophoresed blood was obtained from the ECP machine prior to the initiation of ECP at baseline, and at the 2, 4 and 6 months post treatment time points to assess lymphocyte subsets and cytokine assays. Patients underwent ECP treatments twice on two consecutive days every week for the first four weeks and then every other week for the next 6 months. A comprehensive assessment of organ system involvement using NIH Consensus response assessment tools[1] was done at study entry and at 2, 4 and at six months. Patients were classified as having improvement, stable or progression of cGvHD at 6 months.

Results: Throughout the study, patients with a higher CD4:CD8 ratio responded more favorably to ECP therapy than patients with a lower CD4:CD8 ratio. Of the 40 cytokines that trended upwards in responding patients, IL-9, IL-13, IL-10, IL-4,

and TNF · reached statistical significance. After six months, IL-3, IL-4, and IL-5, components of the allergy response have a strong correlation with high production correlating with successful therapy. By contrast, IL-8, RANTES, PDGF-BB, and EGF show a strong negative correlation with successful therapy, particularly at the four-month time.
Conclusion: Our pilot study suggested that the lymphocyte phenotype (high CD4/CD8 ratio) is a useful predictive marker for successful ECP therapy. The five cytokines that strongly correlate with ECP responsiveness contain a mixture of anti-inflammatory (IL-4, IL-10, IL-13) and pro-inflammatory (IL-9 and TNF ·) cytokines, consistent with the potential for cell-mediated and humoral-mediated immune responses. Thus in future studies, patients with low CD4/8 ratios could get alternative therapies in combination to ECP as they will most likely not respond to ECP alone. In addition the response of a patient to ECP could be monitored by their cytokine profile which may predict response ahead of clinical changes.
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P543

Extracorporeal Photopheresis for Treatment of Bronchiolitis Obliterans Syndrome following Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Bronchiolitis obliterans syndrome (BOS) is a rare but life threatening late pulmonary complication after allogeneic hematopoietic stem cell transplantation. Extracorporeal photopheresis (ECP) has shown efficacy in treatment of chronic graft-versus-host disease (cGvHD), as well as in BOS.
Materials (or patients) and methods: Here, we present a retrospective single-center evaluation of ECP in the setting of BOS. 19 patients with mild (2), moderate (13) and severe (4) BOS, according to the NIH Consensus Criteria, were diagnosed between 2004 and 2012 at our institution. Underlying disease was acute myeloid leukemia (AML) and myelodysplasia (MDS) in 11 patients, acute lymphoblastic leukemia (ALL) in 2, myeloma in 3 and myelofibrosis in 3 patients. 7 patients received allo-HSCT from an HLA-identical sibling, 8 patients from an HLA-identical unrelated donor, 1 patient from a mismatched sibling (1 antigen), 1 patient from an haploidentical donor and 2 patients from a mismatched unrelated donor (1 antigen). Onset of cGvHD was de novo in 5, quiescent in 4 and progressive in 10 patients, respectively. Patients had received 1 to 5 (median 4) treatment lines including steroids, mycophenolate mofetil, calcineurin inhibitors, sirolimus, beta-2-mimetics and macrolides. Manifestation of BOS was detected in median 11 months (range, 4-25) after transplantation. ECP was initiated for stabilization of rapidly declining lung function in patients with severe BOS, as well as for improvement of lung function in patients with stable but unsatisfactory results. Improvement of pulmonary function and clinical symptoms, steroid tapering and outcome were analyzed retrospectively. FEV1 and FEV1/FVC were monitored continuously during treatment (every 3 to 6 months). Improvement of lung function was defined as 10% increase of FEV1 from ECP start to last follow up, stabilization as less than 10% increase or decrease of FEV1, and progression as FEV1 decrease of at least 10%. One aim of ECP treatment was to significantly decrease (at least 50%) the dose of steroids during treatment.
Results: After a median of 19 applied ECP cycles (range, 4-56) over a period of 2 to 75 months (median 16 months) 4 patients (21%) showed improvement, 7 patients (37%) stabilization of lung function parameters and 8 patients (42%) progression of BOS in pulmonary function tests, respectively. In addition, 13 patients (68%) tolerated a steroid decrease of at least 50% during ECP treatment. 8 patients (42%) reported clinical improvement independent from results in pulmonary function tests. After a median follow up of 28 months (range, 5-86) from diagnosis of pulmonary GvHD estimated overall survival (OS) after one year and two years was 84% and 63%, respectively. Median OS was 48 months (range 19-86). With 9 patients (47%) surviving causes of death in 10 deceased patients were progression of BOS in 5, relapse in 2, infectious complications in 1, progressive liver GvHD in 1 and unknown in 1.
Conclusion: BOS and cGvHD were confirmed as life threatening complications after allogeneic allo-HSCT. ECP appears to represent a valuable therapeutic option, allowing tapering of steroids and resulting in clinical improvement of symptoms. Verification in a prospective study is necessary to assess the real benefit of ECP in BOS.
Disclosure of Interest: None declared.

P544

Demographic and clinical characteristic of female patients with genital cGVHD

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Introduction: Female genital chronic graft-versus-host disease (fcGVHD) is an under-recognized and undertreated complication impacting quality of life of patients. It is reported to occur in a quarter of long-term female survivors. To determine clinical manifestations and their relation with severity of cGVHD in female patients with genital manifestations we prospectively analyzed a cohort of adult females who were enrolled on the cross-sectional cGVHD natural history study.
Materials (or patients) and methods: A total of 130 consecutive female adult patients with cGVHD were assessed on this NCI protocol (clinicaltrials.gov identifier: NCT00331968) from 2004. to 2014. g. All patients were evaluated by an interdisciplinary team during four days visit. Detailed history, physical examination and laboratory analysis were done. Diagnosis of cGVHD was made according to NIH Consensus Group Criteria. NIH grading criteria was used to determine global cGVHD score as a measure of cGVHD severity. History related to genital issues, gynecology examination and special questionnaire related to demographic parameters, genital symptoms, hormonal therapy and sexual activity were performed. Diagnosis of genital cGVHD was made according to Stratton-Turner criteria. Descriptive statistics and Chi-square test for dichotomous variables were used in the statistical analysis. A P value $<0,05$ was considered to be statistically significant
Results: Most frequent reason for allogeneic stem cell transplantation were acute lymphocitic leukemia, acute

myeloic leukemia or myelodisplastic syndrome which were found in 66 (50.8%) patients. Myeloablative conditioning was performed in 70 (53,8%) patients. In 105 patients (80,8%) peripheral stem blood cells (PSBC) were used as stem source. Median age was 44.5 (range 18-70). Global cGVHD was severe in 97 (74,6%) and moderate in 32 (24,6%) patients. All patients had other organs involved. At the time of evaluation, 84 (64,6%) patients had genital cGVHD. 53 (40,8%) patients with genital cGVHD had severe genital changes (vaginal adhesions, shortened vagina). More than one third of patients with genital cGVHD (34.5%) did not have gynecological symptoms suggestive of genital cGVHD. There was no difference in age, frequency of myeloablative conditioning or cell source between patients with and without genital cGVHD. Patients with severe genital cGVHD were more likely to have severe global cGVHD score ($P = 0{,}035$).

Conclusion: Genital cGVHD should be considered and gynecologic assessment performed in women with cGVHD even if they are asymptomatic. In this large series more than one third of women with genital cGHVD did not have any genital symptoms. Patients with severe global cGVHD stage are more likely to present with genital manifestations and deserve increased vigilance in evaluations.

Disclosure of Interest: None declared.

P545

Importance of gynecological examination in female patients with chronic graft versus host disease

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Introduction: Chronic Graft-Versus-Host Disease (cGVHD) is multisystemic disease and major complication after allogeneic hematopoietic cell transplantation. Although genital cGVHD is one of the frequent manifestations of cGVHD, it is still not well investigated. The goal of this study was to assess prevalence of genital cGVHD and genital human papillomavirus infection (HPV) in female patients with cGVHD.

Materials (or patients) and methods: A multidiscipinary cGVHD team was established at the University Hospital Center Zagreb in collaboration with the National Cancer Institute, National Institutes of Health. From 2013 to 2014, eight adult female patients were examined by a gynecologist skilled in genital cGVHD assessments as part of the protocol ''Clinical and biological factors determining severity and activity of cGVHD after allogeneic hematopoietic cell transplantation''. NIH grading criteria was used to determine global cGVHD score as a measure of cGVHD severity. An extensive hystory, physical and laboratory analyses were obtained at study entry. In all patients, cervical cytology, human papillomavirus testing, vaginal swab for bacterial and fungal colonisation were done. Diagnosis of genital cGVHD was made using Stratton-Turner criteria. HPV were detected by polymerase chain reaction (PCR). Blood was collected to determine the level of luteinizing hormone, follicle stimulating hormone and estradiol in order to define menopause.

Results: Three patients (37.5%) underwent myeloablative conditioning. In 5 patients (62.5%) peripheral stem blood cells (PSBC) were used as stem source. Median age was 43.5 (range 23-57). Global cGVHD was severe in 6/8 (75%) and moderate in 2/8 (25%) patients. All patients had other organs involved. At the time of evaluation, 4/8 (50%) patients had genital cGVHD. All 4 patients with genital cGVHD had severe genital changes (vaginal adhesions, shortened vagina). Gynecological symptoms suggestive of genital cGVHD were reported by all affected patients. Cervical cytology was normal in 8/8 (100%) patients. HPV infection was observed in 3/8 (37,5%) patients, two without genital cGVHD and one with genital cGVHD. Menopausal status was noted in 7/8 (88%) patients. All menopausal patients without genital cGVHD reported dryness and had signs of urogenital atrophy. One patient with vaginal shortening to depth of 5 cm suggesting genital cGVHD but who lacked signs of active genital cGVHD was initiated on dilator therapy. Topical estrogen cream and 4% topical lidocaine was applied to the dilator to reduce pain during dilation. In two months, the vaginal depth was successfully increased from 5 to 12 cm.

Conclusion: In this small series of women with global cGVHD severity classified as moderate to severe, the prevalence of severe vaginal genital cGVHD was 50%. All women with genital cGHVD had vaginal adhesions or shortened vagina. Dilator therapy with topical estrogen cream and topical 4% lidocaine successfully improved vaginal shortening attributed to cGVHD in a patient without signs of active genital cGVHD. Patient without genital cGVHD who reported symptoms of vulvar/vaginal dryness and with signs of urogenital atrophy should be treated with topical estrogen. HPV infection is frequently present in women with cGVHD. Genital cGVHD should be considered and gynecologic assessment with genital HPV and cervical cytology testing performed in all women with cGVHD.

Disclosure of Interest: None declared.

P546

Abstract Withdrawn

P547

Strategy on the Treatment of Steroid-Refractory Severe acute Graft-versus-Host Disease

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Introduction: Acute graft versus host disease(aGVHD) is due to one of the most important causes of treatment related mortality after allo-HSCT. II°-IV° aGVHD rate was 40%(10% > 80%) after allo-HSCT, in which about 30% > 60% of patients suffered from steroid-refractory severe (III°-IV°) aGVHD. Only 10% > 30% of patients could achieve complete remission and long-term survival. We used CD25 monoclonal antibody and tumor necrosis factor receptor-antibody fusion protein for steroid-refractory severe aGVHD, observe the safety and efficacy of the treatment. Ultimately the strategy could reduce aGVHD related mortality and improve long-term disease-free survival in transplantation patients.

Materials (or patients) and methods: 56 patients with steroid-refractory severe aGVHD were studied retrospectively from September 2001 to October 2013. 27 cases during the period from September 2001 to September 2009 were treated with traditional second-line aGVHD therapy, such as high dose of methylprednisolone,CD3 monoclonal antibody, budesonide, FK506, MTX, CD25 monoclonal antibody, plasmapheresis (control group). 29 cases since October 2009 were treated with CD25 monoclonal antibody and tumor receptor necrosis factor TNF-α receptor-antibody fusion protein (combined treatment group). We evaluate the safety and efficacy of the tow types of therapy.

Table 1. Second line treatment response of severe aGVHD			
	combined group	*control group*	P *value*
OR	26/29 (89.7%)	12/27 (44.4%)	<0.001
CR	23/29 (79.3%)	8/27 (29.6%)	<0.001
Organ response			
Skine (CR)	28/28 (100%)	23/23 (100%)	1.000
Liver (CR/PR)	20/21 (95.2%)	4/11 (36.4%)	0.001
GI (CR/PR)	24/27 (88.9%)	9/22 (40.9%)	<0.001

Transplantation related mortality (TRM), relapse rate, overall survival and disease free survival were evaluated with Kaplan-Meier curves.

Results: 56 cases of steroid refractory aGVHD patients achieved hematopoietic reconstitution, and engraftment evidence (STR) showed complete donor chimerism 3-4 weeks after allo-HSCT. 26 cases(89.7%) in combined treatment group had experienced response, of which 23 cases (79.3%) achieved complete remission (CR). 16 cases (55.2%) is alive till now, median survival time was 21.5 months (6-51 months). One-year and four-year OS rate were 60.8% and 51.0%, respectively. One-year and four-years DFS rate are the same as OS. 12 cases (44.4%) in the control group had experienced response, of which 8 cases (29.6%) achieved CR. 4cases (14.8%) is alive till now, median survival time was 67.5 months (62-68 months). One-year and four-year OS rate were 25.9% and 14.8%, respectively. One-year and four-year DFS rate were 22.2% and 11.1%, respectively. One-year and four-year TRM rate were 32.2% in combined treatment group. One-year and four-year TRM rate were 74.1% and 82.7%, respectively, in the control group.One-year and four-year relapse rate were 10.0% and 24.5%, respectively, in combined treatment group. One-year and four-year relapse rate were 14.3% and 35.7%, respectively, in the control group.

Conclusion: CD25 monoclonal antibody combined with TNF-α receptor-antibody fusion protein as the second-line therapy for steroid-refractory severe aGVHD was safe and effective. It effectively increased the CR rate, reduced transplant related mortality, and do not increase the relapse rate of malignant disease. If used in early period of the steroid-refractory severe aGVHD, It could improve the long-term overal survival rate and disease free survival rate.

Disclosure of Interest: None declared.

P548

Association between high uric acid levels and severe acute graft-versus-host disease after reduced intensity conditioning allogeneic stem cell transplantation

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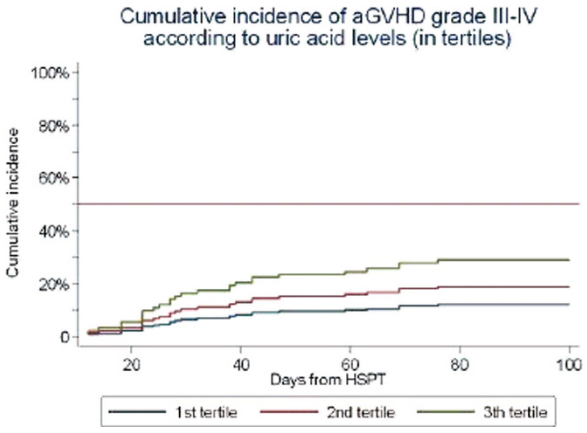
Introduction: There is increasing knowledge on the relevance of danger signals activating the innate immune system in the pathogenesis of GVHD. Uric acid (UA) is a known danger signal that is released from injured cells during conditioning for allogeneic stem cell transplantation (alloSCT). UA has been identified to enhance dendritic cells maturation and amplify T-cell responses. The objective of this study is to investigate the association between UA levels pre-alloSCT and acute GvHD.

Materials (or patients) and methods: We retrospectively analyzed data from 139 consecutive patients who received reduced-intensity conditioning regimen alloSCT from January 2007 to July 2014 on a single institution.

Results: Median age of the patients was 55 years (range 20-67); 58% of them were male. Patients were transplanted for acute leukemia (36%), lymphoma (20.1%), myelodisplastic syndrome (16.5%), multiple myeloma (12.9%) or other (14.4%). Fifty-four percent were transplanted from unrelated donors and 18.7% from HLA mismatched donor. The source of stem cells was peripheral blood in most patients (93.5%). All conditioning regimens were fludarabine-based. Most patients (92.8%) received immunossupression with cyclosporine A in combination with mycophenolate mofetil. Seventeen percent also received ATG. All patients received allopurinol 300 mg/day through conditioning regimen until day 0 of transplant. The median serum UA level prior to stem cells infusion was 3.8 mg/dL (range 0.1-14.5). For analysis, patients were grouped in tertiles according to UA levels: <3 mg/dL ($n=46$); 3-4.5 mg/dL ($n=48$); and >4.5 mg/dL ($n=44$). No relationship was observed between UA levels and development of GVHD grades II-IV. Then, we analyzed the association between UA levels and severe GVHD (grades III-IV). The cumulative incidence of severe acute GVHD was 19% at day +100. In univariate analysis, the development of severe acute GVHD was significantly associated with HLA mismatched (HR 4.1, $P=0.022$) and high (>4.5 mg/dL) UA levels (HR 1.6, $P=0.041$) (Figure 1). No statistical association was observed between GVHD (grades II-IV or III-IV) and recipient sex, hematologic disease, conditioning regimen, GVHD prophylaxis (ATG yes/no), and type of donor (sibling vs. unrelated). In multivariate analysis, only UA level remained a significant predictor for GVHD grades III-IV (HR 1.6, $P=0.032$).



Conclusion: Patients who developed GVHD grades III-IV had a higher level of serum UA during the pretransplantation period compared with those who did not. These results are consistent with the hypothesis that UA could be an endogenous danger signal involved in GVHD pathogenesis. Reducing UA levels prior to day 0 of alloSCT may play an important role in the prevention of acute GvHD.

Disclosure of Interest: None declared.

P549

High erythrocyte-derived microparticle counts at engraftment are a predictive biomarker for graft versus host disease in the reduced intensity conditioning setting

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Introduction: Graft versus host disease (GVHD) is the main limitation of allogeneic HSCT. Currently, there are no validated diagnostic or predictive biomarkers for acute or chronic GVHD routinely used in clinical practice. Microparticles (MP) are being increasingly recognized as regulators of cell-to-cell interactions and are implicated in the pathogenesis of several autoimmune disorders. Circulating MPs are released from cells upon cellular activation or apoptosis. Such MPs may serve as

carriers of autoantigens and may play a role in GVHD pathogenesis. To address this hypothesis, we prospectively studied whether MP derived from different cell sources could serve as predictive biomarker of GVHD in HSCT.
Materials (or patients) and methods: Forty-eight patients submitted to allogeneic HSCT for hematological disorders were included. MP level measurement was performed at engraftment. Patients with graft failure were not included. Annexin V and Trucount beads were used to identify and quantify total MPs (TMPs) by flow citometry. Markers of platelet-derived MPs (PMPs) and erythrocyte-derived MPs (EMPs) were CD61 and CD235a, respectively.
Results: Median age was 41 yrs (2–73) and 69% were males. Diagnosis was acute leukemia in 50%, Hodgkin lymphoma in 18%, MDS in 12%, Non-Hodgkin lymphoma in 9% and benign disorders in 12%. The donor was a full-matched sibling in 27%, a haploidentical relative in 31%, a matched unrelated donor in 21% and umbilical cord blood in 21%. Median follow-up was 18 months (3-28). In patients who developed acute GVHD ($n = 17$), median TMPs, EMPs, and PMPs were not significantly different as compared to patients without acute GVHD (623.1/μl vs. 392.4/μl; 403.9/μl vs. 160.9/μl and 59.5/μl vs. 71.9/μl, respectively). In patients who developed chronic GVHD ($n = 7$), median EMP was higher than in those who did not develop chronic GVHD (447.5/μl vs. 163.3/μl, $P = 0.04$) while TMPs and PMPs were not significantly distinct. The use of myeloablative conditioning regimen was significantly associated with higher EMP (356.65/μl vs. 152/μl, $P = 0.04$) and TMP counts (785.75/μl vs. 350/μl, $P = 0.04$). Selecting only patients submitted to reduced intensity conditioning (RIC, $N = 31$), EMPs were higher in patients who developed acute and chronic GVHD (447.5/μl vs. 71/μl, $P = 0.006$; 439.9/μl vs. 75.7/μl, $P = 0.04$, respectively). In RIC HSCT, EMP counts at engraftment above 400/μl predicted a higher cumulative incidence (CI) of acute GVHD at D + 100 (89% vs. 23%, $P = 0.001$) and of chronic GVHD at 18 months (44% vs. 0%, $P = 0.002$).
Conclusion: EMPs counts at engraftment might be used as a predictive biomarker for both acute and chronic GVHD in the RIC setting. A potential role for MP in GVHD pathogenesis deserves further investigation.
Disclosure of Interest: None declared.

P550
STAT-5 phosphorylation as a marker to predict GVHD

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Introduction: Clinically-relevant markers are needed to identify patients at risk for GVHD after allogeneic hematopoietic stem cell transplantation (aHSCT). We performed a prospective study in 16 adults undergoing aHSCT measuring the frequency of STAT5 phosphorylation (pSTAT5) level, after interleukin 7 (IL-7) stimulation, in $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD3^+CD4^-CD8^-$ T-cells sampled between 1 and 12 months after HSCT.
Materials (or patients) and methods: The level of IL-7-induced STAT5 phosphorylation in immune cell subsets was compared to the constitutive phosphorylation level and to IL-2-induced pSTAT5. Immune cells, longitudinally sampled at 1, 2, 3, 6 and 12 months after aHSCT from 16 patients, were subjected to pSTAT5 analysis. Following overnight resting in serum-free medium, PBMCs were either stimulated with IL-7 or IL-2 and tested by flow cytometry for pSTAT5 using an intracellular staining protocol. We correlated the frequency of pSTAT5 + cells response with CD127 (IL-7 receptor) frequency for each timepoint in association with the clinical events after aHSCT.
Results: We identified a correlation between clinical outcome regarding acute and chronic graft-versus-host disease (aGVHD and cGVHD), and the ability to respond to IL-7 defined by STAT5 phosphorylation. Patients with acute or chronic GVHD had significantly higher frequency of IL-7-induced pSTAT5 + cells as compared to patients without GVHD. PBMCs from patients with or without GVHD did not exhibit differences concerning the frequency of T-cells showing constitutive STAT5 phosphorylation. Patients with aGVHD exhibited a significant higher frequency of IL-7 induced pSTAT5 + $CD3^+CD4^+$ cells at 2 and 6 months after aHSCT ($P = 0,04$ and $P = 0,05$) and at 2 ($P = 0,03$) and 6 months ($P = 0,04$) after aHSCT among $CD3^+CD8^+$ T-cells. The $CD3^+CD4^-CD8^-$ T-cells had a similar profile at 6 months ($P = 0,03$) after aHSCT with a higher frequency of pSTAT5 + cells in patients with aGVHD. Patients with cGVHD exhibited a significant increase in frequency of IL-7-induced pSTAT5 + immune cells as compared to patients without GVHD: in $CD4^+$ T-cells, higher frequency of pSTAT5 was observed during the first 3 months after aHSCT ($P \leq 0,05$). Also the pSTAT5 + $CD8^+$ T-cell population showed a higher frequency upon IL-7 stimulation of blood from patients with cGVHD at 1 ($P < 0,01$), 3 ($P = 0,02$) and 6 months ($P = 0,04$) after aHSCT. We found a higher frequency of pSTAT5 + in $CD4^-CD8^-$ T-cells in patients with cGVHD as compared to the patients who did not develop any GVHD at late timepoints after aHSCT (at months 6 and 12, $P \leq 0,03$).
Conclusion: The increased immune responsiveness to IL-7, reflected by pSTAT5 in different T-cell subsets ($CD4^+$, $CD8^+$ and $CD4^-CD8^-$) from peripheral blood, may represent a clinically-relevant functional biomarker for individuals at increased risk for GVHD: These data may help to offer earlier intervention in preventing GVHD development after stem cell transplantation without increasing the risk for CMV reactivation.
Disclosure of Interest: None declared.

P551
Correlation between serum uric acid levels and cumulative incidence of acute Graft versus Host Disease in a series of 361 patients from a single institution

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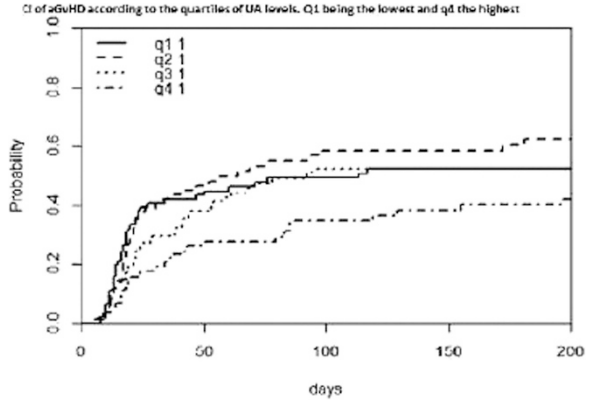
Introduction: Uric Acid (UA) functions as a danger signal and has been previously associated with the pathogenesis of Graft versus Host Disease(GvHD). Recently, a correlation between low uric acid levels and increased risk of grade 2-4 acute GvHD (aGvHD) was described. This was observed only in HLA fully matched related and unrelated allogeneic hematopoietic stem cell transplant (HSCT) patients not receiving Antithymocyte-globulin (ATG). We investigated this association in patients transplanted at our center.
Materials (or patients) and methods: Between 2005 and 2009, 361 consecutive patients with malignant underlying diseases were transplanted with peripheral blood stem cells from matched related (%23), matched unrelated (%44), mismatched related (%1) or mismatch unrelated donors (%32) after receiving myeloablative (%37) or reduced intensity (RIC)(%63) regimens.Acute GvHD was scored according to modified Glucksberg criteria. Patient characteristics are shown in Table 1. GvHD prophylaxis included cyclosporine combined with either mycophenolic acid or methotrexate. ATG dose was 3x10, 3x20 and 3x30 mg/kg for matched related, matched unrelated and mismatch unrelated, respectively. UA levels at day 0 were categorized into quartiles. Cumulative incidences (CI) of aGvHD grade 2-4 were compared between the groups.
Results: Median UA levels were 2.7 mg/dl (range, 0.5-10.7 mg/dl). CI of aGVHD grades 2-4 of patients in the 1st, 2nd, 3rd and 4th quartiles of the UA distribution was 49.2 ± 5.6%, 58.5 ± 6.1%, 52.3 ± 6.0%, and 34.6 ± 5.7% ($P = 0,039$), (figure 1). Furthermore, a Kruskal-Wallis test revealed a significant difference between the UA serum levels at the day of transplantation when the group of patients experiencing aGvHD grades 2-4 was compared with the combined group of patients experiencing aGvHD grade

1 or no aGvHD ($P = 0.041$).We could not find any influence of the use of ATG in our cohort.

age (median in years (range))	*54 (18 - 75)*
sex: male/ female	204(%67)/ 157(%33)
disease risk group: standard / high	177(% 64)/184(% 36)
conditioning regimen: RIC / myeloablative	229(% 63)/132 (% 37)
donor: matched related /unrelated	82 (% 23),158(% 44)
mismatch related /unrelated	3 (% 1), 117 (% 32)
sex mismatch: standard/ high	308 (% 85)/ 53 (% 15)
ATG: no/ yes	65 (% 18)/ 296 (% 82)
aGvHD (overall grade): 0-1/ 2-4	201(%56)/ 160 (%44)



Conclusion: Our findings are consistent with the previous study that reported correlation of low UA levels with CI of aGVHD 2-4. In contrast to that report, we observe this correlation in the entire patient population. Confounding factors deserve further analysis. As a cheap, readily available test, UA merits investigation in more detail as a potential biomarker for aGvHD.
Disclosure of Interest: None declared.

P552
CCR7 expressing naïve and central memory T cells contain the alloreactive cells responsible for the graft-versus-host disease

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Introduction: Graft-versus-host disease (GVHD) represents a major complication of allogeneic stem cell transplantation which is mediated by the migration of donor naïve lymphocytes into the secondary lymphoid organs of the recipient. CCR7 and CD62L are homing molecules characteristically expressed by naïve and central memory lymphocytes and are involved in the normal lymphocyte recirculation. With this background, we aimed to study the involvement of CCR7 and CD62L expressed by the donor lymphocytes in the development and severity of GVHD.
Materials (or patients) and methods: This single center study included 98 donor-recipient pairs. Samples were collected prospectively from the apheresis product and phenotyped by flow cytometry. CD62L and CCR7 expression in CD4+ and CD8+ T-cells were compared between patients who developed acute ($n = 40$) or chronic GVHD ($n = 33$) and those who did not ($n = 38$). Functional assays included both in vitro migration experiments with transwell chambers and activation experiments by quantifying the levels of CD40L and CD69 expression after stimulation with OKT-3 and anti-CD28 antibodies.
Results: The patients who developed acute GVHD were transplanted with a higher percentage of CCR7+CD4+ T-cells ($P = 0.05$) compared to the no GVHD group. These results were confirmed when these patients were divided in degrees according to the severity of the disease; the more severe disease, the higher percentage of CCR7+CD4+ T-cells. Conversely, chronic GVHD patients received a higher percentage of CCR7+CD8+ T-cells ($P = 0.02$) in comparison to those who did not develop the complication. These data were also confirmed when patients were subdivided in degrees of the disease severity. A multivariable analysis confirmed that percentage of CCR7+CD4+ T-cells is a predictive factor of acute GVHD whereas the percentage of CCR7+CD8+ T-cells is a predictive factor of chronic GVHD.
In vitro migration experiments demonstrated that donor T-cells, which were infused into patients who developed GVHD, had a higher migratory capacity in response to the ligands of CCR7, CCL19 and CCL21, when compared to the migration of those donors whose recipients did not develop GVHD. Moreover, a higher percentage of CD4+ T-cells migration was observed in those patients who developed acute GVHD compared to those who developed chronic GVHD. Conversely, the donor CD8+ T-cells seemed to migrate more in the chronic group than in the acute one. The role of CCR7+ T-cells in the pathogenesis of GVHD was further confirmed by activation assays showing that CCR7+ T-cells activated more in response to in vitro stimulus than the CCR7 negative T-cells.
Conclusion: Our prospective study indicates that the CCR7+ T-cells from the donor, which include naïve and central memory T-cells, contain the alloreactive cells with a high ability to mediate GVHD (in the case of both migration and activation). We therefore suggest that the proportion and functional properties of CCR7+CD4+ and CCR7+CD8+ T-cells in the apheresis could act as a predictive biomarker to both acute and chronic GVHD respectively.
Disclosure of Interest: None declared.

P553
Extracorporeal Photopheresis In Steroid Refractory Pediatric GVHD Patients: A Single Center Experience

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Introduction: Graft versus host disease is one of the biggest concern experienced after hematopoetic stem cell transplantation. Mortality is high particularly in steroid refractory patients (1). During recent years, some improvements have developed in treatment modalities, which the extracorporeal photopheresis (ECP) stands for a promising alternative in steroid refractory or dependent patients (2,3). However, experience with ECP in children is limited. The aim of our study was to present our experience in ECP performed steroid refractory pediatric acute and chronic GVHD patients.
Materials (or patients) and methods: ECP was performed in 26 pediatric patients who developed steroid refractory GVHD after allogeneic transplantation in Bahçeşehir University, Medicalpark Antalya and Göztepe Hospitals Pediatric Hematopoetic Stem Cell Transplantation Units in Turkey. HSCT was performed for malignancy in 13 patients and also for 13 patients who have a non-malignant disease. The demographic and clinical data of GVHD recorded. Response to ECP evaluated with the degree of downgrading of the stages of the GVHD.
Results: Age and weight range were 11 months-17 years and 7-68 kg, respectively. There was skin, liver and gastrointestinal involvements in 16, 4 and 15 patients, respectively. The number of the organ involvements were 1 for eight patients, 2 for eleven patients, 3 for six patients, and five for 1 patient. At the beginning of the ECP, acute GVHD (aGVHD) existed in 13 patients and chronic in 8 patients while five patients had overlap syndrome. The median initial ECP administration time after HSCT and after the beginning of GVHD were 9 and 3,5 weeks, respectively, for aGVHD and 44 and 20 weeks, respectively, for

cGVHD. One patient started ECP 12 years after the HSCT because of her scleromatous skin. On the clinical assessments at the last visit, aGVHD downgraded 1 stage in seven patients, 2 stages in five patients and 4 stages in one patient in skin GVHD, while there was no response in three patients; 2 stages in two patients, 3 stages in six patients and 4 stages in two patients in GIS GVHD, while there was no response in five patients; hepatic GVHD did not responde in any stage. The severity of chronic GVHD (cGVHD) downgraded from severe to moderate in seven patients. There was no improvement in five patients in cGVHD. Seven of twentysix patients died during ECP, four for nonresponsive GVHD, one for mucormicosis, one for sepsis and one for catheter related infection.

Conclusion: In this preliminary assessment of ECP performed pediatric patients, we observed better response in most of the patients. The response was better in skin involvements. There was no significant clinical and biochemical problem happened during procedures even in low-weight patients. We think that, data about the efficiency and safety of ECP would strengthen up by increasing the number of the patients by a longitudinal follow-up.

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Disclosure of Interest: None declared.

P554

Atorvastatin for the Prophylaxis of Acute Graft vs. Host Disease (aGVHD) in Patients with Hematological Malignancies Undergoing HLA-Matched Related Donor Allogeneic Hematopoietic Stem Cell Transplantation (allo-SCT)

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Introduction: Acute GVHD (aGVHD) is a leading cause of morbidity and mortality following allo-SCT. The statin class of cholesterol lowering medications can reduce pro-inflammatory cytokines, increase anti-inflammatory cytokines and stimulate T-regulatory and T_h-2 helper cells, holding promise for prevention of aGVHD. A single institution prospective phase II study showed that the addition of atorvastatin to matched sibling donors prior to stem cell collection and to recipients as aGVHD prophylaxis resulted in day 100 and 180 aGVHD incidence of only 3.3% and 11.1% respectively(Hamadani,J Clin Oncol, 2013). We present the results of our phase II clinical prospective trial evaluating essentially the same atorvastatin-based strategy for the prophylaxis of aGVHD in HLA-matched related donor allo-SCT

Materials (or patients) and methods: Forty patient were enrolled (NCT01491958) between Mar/2012 and Jan/2014. Atorvastatin 40 mg/day orally was administered to sibling donors, starting 14-28 days before the anticipated 1st day of peripheral blood (PB) stem cell collection. In allo-SCT recipients, atorvastatin (40 mg/day) was administered from at least day -7 to day +180 (or until stopping rules). In addition all recipients received standard GVHD prophylaxis with tacrolimus and methotrexate. Primary outcomes were rate of Gr 2-4 aGVHD at day +100 and safety of atorvastatin administration to allo-SCT donor/recipient pairs. The study was designed to test the null hypothesis H_0: $P \geq 35\%$, vs. the alternate H_1: $P \leq 15\%$, where p is probability of Gr 2-4 aGVHD at day 100. We compared our defined endpoints to 96 patients undergoing matched sibling PB allo-SCT between Aug/2006 and Feb/2012 where neither the patient nor the matched donor received any cholesterol-lowering medications (controls)

Results: Median patient's age was 51 yrs.(range 27-71) and of donors 50 yrs.(range 25-68). Intermediate/high risk disease constituted 57.5% of patients by ASBMT criteria and 90% by disease risk index. Twelve patients received MA regimen. Median duration of atorvastatin in donors was 14 days (range 13-27) and in patients 132 days (range 32-400) and was well tolerated with no Gr 2-4 adverse events attributable to atorvastatin. Day 100 and 180 cumulative incidences of grade 2-4 aGVHD were 30% (95% CI 17- 45%) and 43% (95% CI 21-70%) respectively. One-year cumulative incidence of cGVHD was 43% (95% CI 32- 69%), non-relapse mortality 5.5% (95% CI 0.9-16.5%) and relapse 38% (95% CI 18- 47%). One-year progression-free survival (PFS) and overall survival (OS) were 54% (95% CI 38-71%) and 82% (95% CI 69-94%). Compared to our matched historic controls, we did not find any significant differences in aGVHD ($P = 0.8303$), cGVHD ($P = 0.9149$), relapse ($P = 0.4311$), PFS ($P = 0.2864$) or OS ($P = 0.0978$) (Figure)

Conclusion: The administration of atorvastatin to allo-SCT sibling donors/recipients appears to be feasible, safe and tolerable. However, we did not find any reduction in the incidence of aGVHD and cGVHD compared to historical

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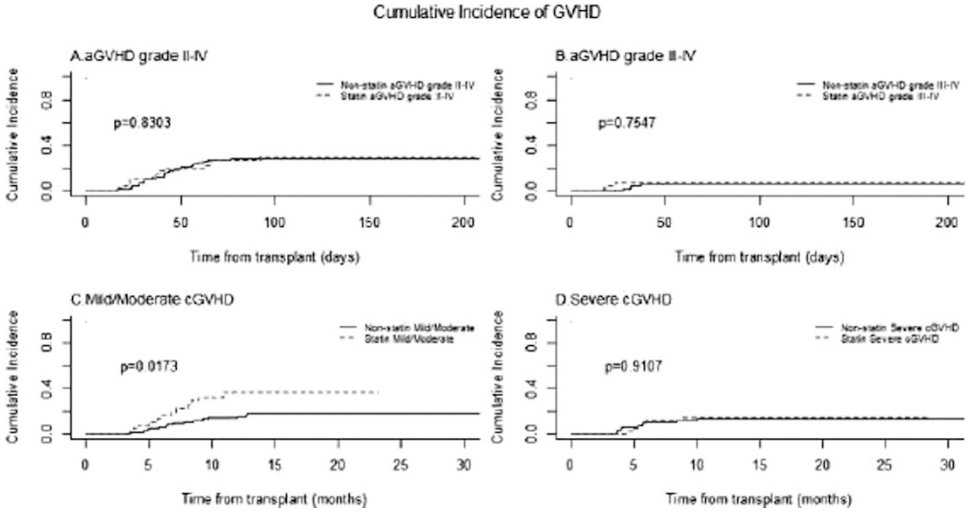

patients not exposed to statins. In addition, our findings support the notion that trials evaluating novel strategies to prevent GVHD should be adequately controlled and involve multiple institutions as early as possible

Disclosure of Interest: None declared.

P555

Impact of Atorvastatin on Immune Reconstitution and cytokines of Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

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Introduction: Donor T cells are known to be the most powerful effectors for acute graft-versus-host-disease (aGVHD). Statins have been shown to reduce pro-inflammatory cytokines, increase anti-inflammatory cytokines, T regulatory (T-regs) and T_h-2 helper cells with a potential to reduce aGVHD. We analyzed the effect of atorvastatin on cytokines and on the immune reconstitution pattern of patients undergoing HLA-matched-related donor peripheral blood (PB) HSCT in which donors and patients were exposed to atorvastatin. The results of this study were compared to historical matched controls where neither donor nor patients were exposed to any cholesterol lowering medications.

Materials (or patients) and methods: Forty patients (statin gp) were enrolled (NCT01491958) between Mar/2012 and Jan/2014. Atorvastatin 40 mg/day orally was administered to sibling donors, starting 14-28 days before the anticipated 1st day of stem cell collection. In HSCT recipients, atorvastatin (40 mg/day) was administered at least day -7 to day + 180 (or until stopping rules). In addition all patients received standard GVHD prophylaxis with tacrolimus and methotrexate. Controls (non-statin gp) were 25 patients who had PB allograft samples (day 0) and PB samples on days 30 and 100 post HSCT collected. We analyzed the absolute numbers of T, NK, NKT, and B cells and the levels of 27 cytokines.

Results: Median duration of atorvastatin in statin donors was 14 days (range 13-26) and in patients 132 days (range 32-400). Age, sex, donor/recipient sex, intensity of regimen, disease status at HSCT and number of stem cells infused were matched between the gps. The clinical results of this study are presented in a separate abstract in which we found no statistically significant difference in clinical outcomes including aGVHD and cGVHD between the two gps. While there were no differences in the B and total T cells reconstitution in all time points, the statin gp allografts contained less NK and T-regs than the non-statin gp (Table). We also found no effect of atorvastatin on 26 cytokines, specifically IFN-γ, TNF-α, IL-2, IL-4 and IL-10 except for a marked elevation in RANKES ($P<0.0001$) in the statin gp, a protein that has been associated with increased GVHD.

Marker Allograft(day 0)	*Percent Median (range) Statin N = 40*	*Percent Median (range) Non-statin N = 25*	*Wilcoxon test P-value*
CD4 + /CD25 + /CD127-	0.10 (0-1.60)	1.0 (0-4.7)	*<0.0001*
CD4 + /CD25 + /CD127-ALT	0.25 (0-1.20)	0.6 (0.2-1.5)	*0.0072*
CD3-/CD5616 + /CD159a +	1.60 (0.20-5.30)	3.4 (0.8-8.3)	*0.0003*
Day 30 CD4 + /CD25 + /CD127-	0.20 (0-2.30)	0.9 (0-3.6)	*0.0010*
CD3-/CD5616 + /CD63 + / CD314 +	0.30 (0-3.40)	2.7 (0.3-17.6)	*<0.0001*
CD16 + /CD56 + /CD3-/CD117-	9.90 (0.70-38.20)	21.6 (3.9-47.8)	*0.0009*
Day 100 CD3 + / CD86 +	0.2 (0-1.3)	0.5 (0.1-1.3)	*0.0158*
CD4 + /CD25 + /CD127-	0.1 (0-0.9)	0.8 (0.1-1.8)	*0.0002*
CD3-/CD5616 + /CD63 + / CD314 +	0.3 (0-6.3)	1.9 (0.2-14.8)	*0.0003*

Conclusion: while we found no differences in clinical outcome and on most cytokine levels with the addition of atorvastatin, it did result in a statistically significant decrease in NK and T-reg cells for the statin gp. The significance if this is yet to be determined.

Disclosure of Interest: None declared.

Infectious complications II

P556

Features of EBV viremia and outcomes in patients received allogeneic stem cell transplantation: a Chinese multicenter survey

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Introduction: EBV reactivation and associated diseases have been well recognized as one of the life threatening complications after SCT, which leads to the establishment of prophylactic and preemptive treatment for EBV viremia. However since then, few epidemic data was shown in SCT recipients, especially in China.

Materials (or patients) and methods: An observational data base study was conducted in patients with hematological disorders who received allo-SCT from Jun 2011 through Jun 2014. EBV-negative status was confirmed before transplant for both donors and recipients. EBV-DNA was screened weekly until Day 100 post SCT by qPCR, and then every 2-4 weeks until 1 year. Additional tests were carried out when clinically indicated. All patients received ganciclovir during conditioning regimen, and then switched to acyclovir or valaciclovir after stem cell infusion until 1 year post SCT. Ganciclovir and/or foscarnet were used when DNAemia developed, as well as rituximab for high risk patients such as high viral load or persistent DNAemia.

Results: This study recruited 892 evaluable cases, including 91 cases of benign diseases and 801 cases of malignancies. EBV-DNA was detected in 165 cases with a median duration of 55 days (16-618days) post SCT, and the long-term cumulative incidence of DNAemia was $21.3 \pm 1.5\%$. Totally 7 patients developed probable or proven PTLD. Log-rank test showed EBV-DNAemia had impact on neither 2 year-OS ($67.2 \pm 6.3\%$ vs $66.2 \pm 1.9\%$, $P = 0.341$) nor 2 year-NRM ($19.4 \pm 4.0\%$ vs $25.1 \pm 1.8\%$, $P = 0.272$). Cox model analysis revealed that haploidentical donor (RR 1.973 [95%CI 1.383-2.814], $P < 0.001$) and the use of ATG/ALG (RR 4.831 [95%CI 2.469-9.524], $P < 0.001$) were independent risk factors (Figure 1). While focusing on leukemia patients, RIC regimen was identified as an additional risk factor (RR 3.378 [95%CI 1.058-10.870]), with a weaker association ($P = 0.040$).

Conclusion: This work showed the cumulative incidence of EBV viremia after allogeneic SCT was around 21.3% based on virus prophylaxis. EBV viremia had no impact on OS or NRM for entire population, and PTLD was rarely observed, which implied the efficacy of preemptive treatment. Haploidentical donor and use of ATG/ALG were independent risk factors, and for leukemia patients, RIC regimen was an additional risk factor.

Disclosure of Interest: None declared.

[P556]

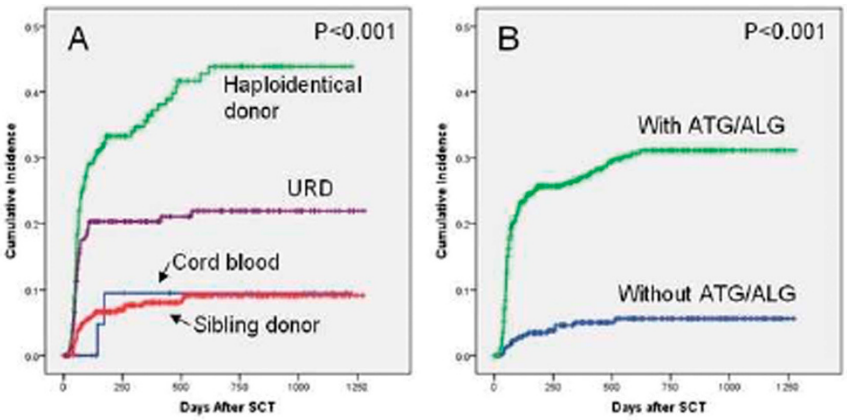

Fig1. Cumulative incidence of EBV DNAemia in entire population: A. Comparison of incidence among different donor types; B. Comparison of incidence between conditioning regimens with and without ATG/ALG

P557

Earlier diagnosis of posttransplant lymphoproliferative disorder (PTLD) after allogeneic stem cell transplant with routine EB virus monitoring and Rituximab containing regimen improved remission rate and PTLD-specific mortality, but not overall survival

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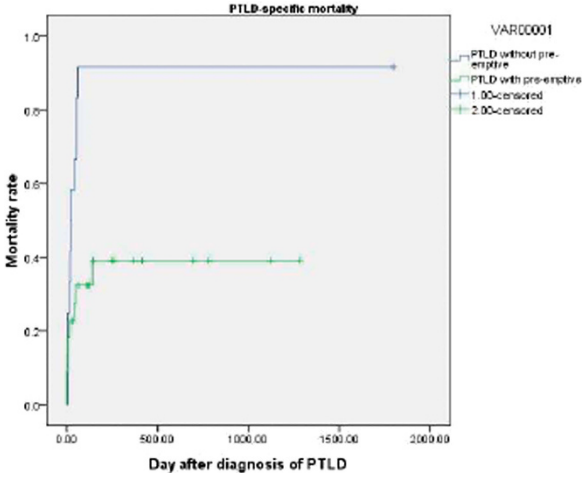
Introduction: Post-transplant lymphoproliferative disorder (PTLD) is one of the major complications after allogeneic stem cell transplantation. We retrospectively analyzed the PTLD diagnosed at our institute in the past 21 years and examined the effect of earlier diagnosis on clinical outcome.

Materials (or patients) and methods: Between Jan 1993 and September 2014, total 34 cases of PTLD among 666 patients after allogeneic hematopoietic stem cell transplant (allo-SCT) were reviewed retrospectively. From Jan 2007, two strategies had been adopted in our hospital: 1) Early detection of EBV viremia or PTLD by routine EBV DNA Q-PCR surveillance after allo-SCT with pre-emptive treatment, and 2) routine use of Rituximab (R) to EBV viremia and R or R-COP/CHOP to PTLD. The outcome of the old ($n = 12$) and new cohort ($n = 22$) were compared. Overall survival probability (OS) and PTLD-specific mortality rate (PTLD-M) were estimated by the Kaplan-Meier method. Multivariate analysis were performed using Cox proportional hazard regression model.



Results: Cumulated incidence of PTLD after allo-SCT at one-year is 2.70%. Within one month of PTLD diagnosis, 70% of patients had CMV reactivation, and 61% had grade II or higher aGVHD. Donors other than match related donor and thymoglobulin use during conditioning regimen were seen in 73% and 94% of patients having PTLD, respectively. Multivariate analysis showed non-match sibling donor and use of antithymocyte globulin (ATG) to be independent risk factor for PTLD. From Jan 2007, after adopting the new strategies for PTLD, we made earlier diagnosis of PTLD with extra-nodal involvement upon diagnosis decreased from 83% (10/12) to 45% (10/22). Routine use of R or R-COP/CHOP for treatment of PTLD increased from 58% (7/12) to 100% (22/22). The above two changes lead to a complete remission (CR) rate of PTLD increased from 8% to 63%. One-year PTLD-specific mortality decreased from 92% to 39% ($P < .01$) (Figure 1). However, OS were not significantly improved ($P = .052$, one-year OS 8% vs 34%). The other causes of death for the new strategies were infection (22%), or relapse (14%).

Conclusion: Routine EBV monitoring earlier diagnosis of PTLD and R or R-COP/CHOP treatment of PTLD significantly increase CR rate and decrease PTLD-related mortality. However, present of PTLD remains a poor prognostic marker for OS after allo-SCT, possibly due to an imbalance of host immunity and the complicated clinical situation while PTLD diagnosed.

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Disclosure of Interest: None declared.

P558

Poor CMV-specific CD8+ T central memory subset recovery at early stage post-HSCT associates with uncontrolled CMV reactivation

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Introduction: Patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) are threatened by potentially lethal viral infections, especially cytomegalovirus

(CMV) reactivation. Despite the pre-emptive therapy relying on strict virological monitoring, refractory and recurrent reactivation still developed in a subgroup of patients, leading to increased CMV disease and mortality.[1] CMV-specific CD8+ T cells with a central memory phenotype (T_{CM}) play an important role in clearing systemic CMV infections and forming life-long immune protection.Even a single antigen-specific CD8+ T_{CM} cell can develop into diverse effective and memory subsets and protect against bacterial challenge.[2] We proposed a possibility that the recovery of CMV-specific CD8+ T_{CM} subset at early stage post-HSCT might impact the reconstitution of CMV-specific T cell immunity post-HSCT and differ between patients with controlled and uncontrolled CMV.

Materials (or patients) and methods: A prospective study in two independent cohorts (training cohort, $n=64$; validation cohort, $n=43$) was conducted. CMV-specific CD8+ T cell were determined monthly, using HLA class I pentamer.

Results: In the training cohort, the patients with lower level of CMV-specific CD8+ T_{CM} subset at day 30 post-HSCT had an increased risk of uncontrolled CMV infection (59.4%) comparing with the higher one (9.3%)($P=0.001$) and poorer CMV-specific CD8+ T cell reconstitution during the first six months post-HSCT ($P=0.037$).(Figure 1) In the validation cohort, the CMV-specific CD8+ T_{CM} subset at day 30 was predictive of uncontrolled reactivation with a sensitivity and specificity of 95.6% and 65%, respectively. Multivariate analysis revealed that CMV-specific CD8+ T_{CM} subset at day 30 was an independent prognostic factor for uncontrolled reactivation in the training ($P=0.003$) and validation cohort ($P=0.003$).

Conclusion: CMV-specific CD8+ T_{CM} subset recovwry at day 30 post-HSCT was associated with CMV-specific T cell immunity post-HSCT and might identify patient with high risk of uncontrolled CMV.

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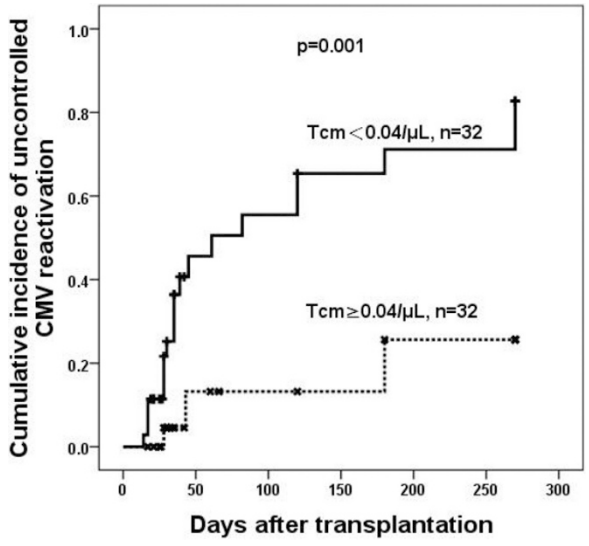
Disclosure of Interest: None declared.



P559

T-cell receptor deep sequencing analysis of EBV specific T cells before and after adoptive transfer in a patient after allogeneic stem cell transplantation

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Introduction: Reactivation of Epstein-Barr-Virus (EBV) after HLA matched allogeneic hematopoietic stem cell transplantation (aHSCT) occurs in up to 30% of patients and frequently requires treatment with Rituximab due to the risk of EBV related lymphoma. Generation of EBV specific T cells by peptide stimulation for the treatment of reactivation is fast and efficient, can be carried under GMP conform conditions and has been shown to be efficient in virus control over a long period of time after transfer. However, it remains unclear which EBV specific T cells survive long term and therefore can be considered the disease controlling effector cells.

Materials (or patients) and methods: Next generation T cell receptor sequencing and flow cytometric analysis of peripheral was applied in the donor and in a patient with severe EBV reactivation before and after adoptive T cell transfer of EBV specific T cells after allogeneic stem cell transplantation.

Results: We show here that by using a peptide pool derived from different early and late EBV proteins multi-epitope EBV specific T cells can be generated in a GMP conform fashion. Using peptide loaded HLA class I multimers for flow cytometric analysis we identified in the peripheral blood of a HLA*A02:01, B08:01, B35:01 positive donor approximately 1% EBV specific $CD8^+$ T cells with specificity for the peptides used in the peptide pool. Donor $CD8^+$ T cells were expanded and enriched by peptide pool stimulation to a total specificity of approximately 60% in the final T-cell product. After adoptive transfer (1.0 Mio. CD3+T-cells/kg bodyweight) EBV specific T cells further expanded in the patient and displayed a significant release of IFNgamma and other proinflammatory cytokines. The expansion was associated with control of EBV reactivation and no further EBv genome was detecable in the peripheral blood after adoptive transfer.

By high-throughput sequencing (HTS) analysis of the T-cell product and of HLA multimer soreted CD8+ T cells we identified approximately 400 different T-cell receptor beta (TCRβ) rearrangements with defined specificity for the EBV peptides used for stimulation. These TCRβ sequences were partially found with low frequencies in the corresponding stem cell donor, but not in the recipient early (day 60) after aHSCT and before adoptive transfer of EBV specific T cells. HTS of $CD8^+$ T cells of the recipient's peripheral blood at several time points after adoptive transfer revealed that only a minority of TCRs found in the T-cell product persist long term in the patient for up to 230 days. In addition, several TCRs found to be dominant in high frequencies in the peptide stimulated T-cell product disappeared over time or were found only in very low frequencies after moire than 200 days.

Conclusion: Our results demonstrate that selection of TCRs by peptide stimulation *in vitro* differs greatly from selection of TCRs *in vivo* in the patient. In addition, only a minority of T cells with distinct TCR's persists long term in the patient and mediate control of latent EBV.

Disclosure of Interest: None declared.

P560

Comparison of characteristics of herpes zoster between recipients of allogenic and those of autologous hematopoietic stem cell transplantation

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Introduction: Herpes zoster (HZ) is a common infectious complication of hematopoietic stem cell transplantation (HSCT), and leads substantial decline of post-HSCT quality of life often. Although allogenic (allo-) and autologous (auto-) HSCT are fundamentally different procedures in immunologic perspectives, there is still a scarce of previous studies that compare the characteristics of HZ according to the setting of HSCT, and current guidelines propose similar recommendations for HZ. Thus, we evaluated and compared the characteristics of HZ between recipients of allo- and those of auto-HSCT, both of them received uniform anti-viral prophylaxis.

Materials (or patients) and methods: A retrospective cohort of recipients who underwent either allo- or auto-HSCT between Jan. 2003 and Aug. 2014 were analyzed. Adult patients with well-preserved clinical and laboratory data were included. Patients with a second HSCT and any episode of HZ during therapy before HSCT were excluded. Prophylactic acyclovir was applied to every patient, but limited from D-7 to D + 30 or discharge (250 mg/m^2 every 12 hours i.v.). Similar prophylaxis for infection [oral ciprofloxacin and one of three azoles (fluconazole, itraconazole, or fosaconazole)] and GVHD (methotrexate plus either cyclosporine or tacrolimus) was applied, and TBI was not used. Incidence of HZ was estimated with Grey's test for the statistical consideration of two competing events; 1) any re-treatment due to relapse, and 2) death from any cause except HZ. Evaluation of potential risk factors was performed using a proportional hazard regression model.

Results: Overall 185 patients (99 patients for all- and 86 for auto-HSCT) were analyzed. Nineteen of allo-HSCT and 21 of auto-HSCT recipients experienced HZ after HSCT. The 6 month, 1-year, and 2-year cumulative incidence of HZ among patients with allo-HSCT was 24.9%, 27.1%, and 36.5%, respectively, and for auto-HSCT recipients 11.1%, 19.1%, and 31.4%, respectively. Among allo-HSCT recipients, none of evaluated potential risk factors (age > 40 years, acute myeloid leukemia, not matched related donor, myeloablative conditioning, higher HCT-CI score, CMV antigenemia, and G ≥ 2 acute GVHD) were related to the incidence of HZ. Fifteen out of the 19 patients (78.9%) experienced HZ during the use of immunosuppressants and/or steroids. Use of gancyclovir after allo-HSCT showed very strong protective effect against HZ: there was no episode of HZ during use of gancyclovir and even within 30 days after gancyclovir discontinuation. Among auto-HSCT recipients, age > 40 years ($P = 0.023$) and multiple myeloma ($P = 0.051$) were risk factors of HZ in both univariate and multivariate analysis. The incidence of HZ during the first 6 months after auto-HSCT was not high as incidence after allo-HSCT, however, the cumulative incidence increased gradually for 2 years after auto-HSCT.

Conclusion: Concurrent immunosuppression was so overwhelming that other risk factors may lose hazard of HZ in post allo-HSCT setting. Use of gancyclovir also had an impact on the incidence of HZ. On the contrary, among auto-HSCT recipients, risk of older age, the strongest risk factor of HZ among general population, was preserved, and patients with multiple myeloma had significantly higher HZ incidence. Establishment of tailored strategies according to distinctive natures of HZ after allo- and auto-HSCT should be considered.

Disclosure of Interest: None declared.

P561

Safety considerations of a new anti-CMV cellular therapy – experience from donor-derived, directly selected, adoptively transferred T cells (Cytovir™ CMV) in 2 randomized clinical trials

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Introduction: One of the major concerns in the adoptive transfer of donor T cells following allogeneic hematopoietic stem cell transplantation (HSCT) is new onset or aggravation of acute GvHD. This risk can be mitigated by depletion of potential alloreactive cells or selective enrichment of antigen-specific T cells. Here we report on the safety of adoptive transfer of MHC class I-selected T cells to prevent or treat CMV reactivation post allogeneic HSCT.

Materials (or patients) and methods: The IMPACT study (NCT01977908) was conducted in patients who received grafts from related donors; adoptive cellular therapy (ACT, Cytovir CMV) was given at a target dose of 5 x 10^4 CD3 cells/kg. The ASPECT study (NCT01220895) was conducted in patients who received grafts from unrelated donors, with Cytovir CMV given at a target dose of 3 x 10^4 CD3 cells/kg. Cytovir CMV was manufactured by direct selection of mobilized or nonmobilized apheresis starting material using Streptamer® binding technology (STAGE Cell Therapeutics GmbH), resulting in a median purity of 92.0% CD3+ CMV-specific cells. Immune reconstitution and clinical efficacy from these studies has been previously reported.

Results: In the ASPECT and IMPACT studies, patients were randomized to antiviral treatment alone or antiviral treatment plus Cytovir CMV. In both studies there was no apparent difference in the incidence of new onset acute GvHD in the Cytovir CMV groups compared to the control (see Table). Serious adverse events were also comparable between the control and the treatment groups, and were typical in severity and frequency for this patient population (data not shown).

Table: Incidence of new onset acute GvHD > grade I following baseline assessment

ASPECT			*IMPACT*		
	Number of patients with GvHD	*Incidence in cases per 1000 days (95% CI)*		*Number of patients with GvHD*	*Incidence in cases per 1000 days (95% CI)*
Standard best antiviral ($n = 11$)	3	2.34 (0.76: 7.27)	Standard best antiviral ($n = 44$)	4	0.65 (0.24: 1.73)
Cytovir CMV + antiviral ($n = 17$)	3	1.30 (0.42: 4.02)	Cytovir CMV + antiviral ($n = 20$)	2	0.70 (0.18: 2.80)

Conclusion: The primary safety concern was the potential for Cytovir CMV to elicit or exacerbate acute GvHD. Cytovir CMV doses were used that were equivalent

to approximately half a log less than the number of unselected T cells typically administered with a T cell-depleted marrow graft, and approximately 0.5 log lower than the minimum dose given in a non-selected donor lymphocyte infusion from an unrelated donor. There was no evidence of an increase in incidence of new onset acute GvHD with Cytovir CMV treatment, and no other safety signals related to Cytovir CMV infusion were detected. In conclusion, the administration of Cytovir CMV, at the doses between 3-5 x 10^4 CD3 cells/kg is safe and supports the use of Cytovir CMV in unrelated and related allogeneic HSCT.

Disclosure of Interest: K. Peggs: None declared, E. Tholouli: None declared, R. Chakraverty: None declared, A. Peniket: None declared, A. Bloor: None declared, E. Nikolousis: None declared, F. Chen: None declared, S. Devereux: None declared, K. Orchard: None declared, C. Crawley: None declared, D. Marks: None declared, A. Parker: None declared, S. Robinson: None declared, G. Cook: None declared, T. Pagliuca: None declared, R. Clark: None declared, K. Thomson: None declared, P. Moss Conflict with: Member of Cell Medica Scientific Advisory Board

P562

Invasive Pulmonary Aspergillosis in allogeneic bone marrow recipients with thalassaemia or sickle cell anaemia: Incidence and Outcome

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Introduction: Invasive Pulmonary Aspergillosis (IPA) is a devastating opportunistic infection and remains a significant cause of morbidity and mortality in allogeneic Haematopoietic Stem Cell Transplantation (HSCT) recipients. IPA has been well characterized in adults and in the setting of oncological transplant. No data are available regarding IPA in patients with β- globin gene disorders undergoing bone marrow transplant (BMT).

OBJECTIVE: To evaluate the incidence and the outcome of IPA among BMT recipients with β-Thalassaemia Major or Sicke cell Anaemia (SCA).

Materials (or patients) and methods: We evaluated the occurrence, the clinical setting and the clinical outcome of IPA in pediatric patients affected by Thalassaemia major or SCA transplanted at our institution.

Results: A total of 276 consecutive patients (median age 9,3, range 11,7-28 years) with β- globin gene disorders who underwent BMT (198 HLA-identical, related donor; 52 haplotype-identical donor, 22 HLA-mismatch, related donor and 4 matched, unrelated donor) were studied. Overall, the incidence of proven or probable IPA was 2.1% (6 out of 276 cases). The median time to onset IPA infection after transplantation was 68 days (range, 13-183 days). In particular, 3 cases (50%) were diagnosed after post-BMT day 60 and 3 (50%) were diagnosed during the post-BMT neutropenic period before engraftment.

Graft-versus-host-disease (GVHD) was present in 5 (83.3%) of 6 patients with IPA, compared with 70 (25.9%) of 270 patients without fungal infection ($P = 0.01$). Among 6 cases with IPA an alternative donor (matched unrelated and haplotype-identical) was used in 3 patients (50.0%) compared with 53 cases (19.6%) of 270 recipients without IPA ($P = 0.06$).

The infection remained confined in the lunge in 5 (83.3%) of 6 IPA cases, in 2 cases surgical intervention was successfully adopted in addition to the adequate systemic anti-fungal medical therapy; only in 1 case the infection was multifocal with CSN involvement. The overall mortality rate for IPA was 0.74% (2 of 276 patients) whereas the IPA attributable mortality rate observed in our population was 33.3% (2 of 6 cases). See the table

Table: Characteristics of patients with and without invasive pulmonary aspergillosis (IPA) after bone marrow transplantation

Variable	*IPA(n = 6)*	*No IPA(n = 270)*	P
Sex, male/female	4/2	157/113	
Mean age, years	13,3 (3-17)	10,2 (1,7-28)	
Beta-talassaemia	5 (83.3%)	210 (77.7%)	n.s.
SCA	1 (16.6%)	54 (20%)	n.s.
Thal/Sca		6 (2.2%)	n.s.
Donor			
matched-related donor	3 (50%)	217 (77.7%)	n.s.
Alternative*	3 (50%)	53 (19,63)	n.s.
GVHD (all grade)	5 (83.3%)	70 (25.9)	0.01

*matched unrelated and haplo-identical

Conclusion: Our data show that in a population affected by β-globin gene disorders undergoing allogeneic BMT, the IPA rarely develop (2,1%) and the IPA attributable mortality rate (33.3%) is markedly lower then the one observed in the setting of haematological malignancies. In our cohort, a significant risk factors for IPA was GVHD.

Disclosure of Interest: None declared.

P563

Cidofovir for severe Adenovirus and BK virus-associated hemorrhagic cystitis in allogeneic hematopoietic stem cell transplantation recipients

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Introduction: Late-onset hemorrhagic cystitis (LHC) is a common viral infections after allogeneic hematopoietic stem cell transplantation (HSCT). Reactivation of adenovirus(ADV), BK virus(BKV), and cytomegalovirus(CMV) infection have been associated with late onset hemorrhagic cystitis(LHC) in HSCT. Most cases of LHC occurring after HSCT are self-limited, but they can cause pain, pollakiuria, and prolonged hospitalization. In cases with progression of LHC, ureteral stenosis has occurred and occasionaly resulted in obstructive renal failure.Cidofovir (CDV) is a monophosphate nucleotide analogue that demonstrates a broad range of antiviral activity in vitro including BKV and ADV, but its use for severe LHC in HSCT recipients has not yet been established as a standard therapy.

Materials (or patients) and methods: We retrospectively analyzed the clinical records of 417 patients who underwent HSCT at Toranomon Hospital between 2010 and 2012. LHC was defined by presence of microscopic hematuria or sustained macrohematuria for more than 7 days with clinical signs of cystitis such as pain, dysuria, increased frequency and other urinary tract symptoms in the absence of other conditions such as gynecologic-related bleeding, multiple organ dysfunction syndrome, or sepsis. To exclude conditioning regimen-related HC, HC occurring more than 10 days after transplant was defined as ''late-onset''.LHC grade was described as suggested by Bedi et al.: 0 = no hematuria, 1 = microscopic hematuria, 2 = macroscopic hematuria, 3 = presence of blood clots, 4 = renal impairment due to urinary obstruction. Hyperhydration with continuous infusions of normal saline and glucose was given to all patients with LHC. Patients with grade 3 or 4 LHC were treated with continuous bladder irrigation with normal saline. Therapy for refractory or life-threatning LHC included cystoscopy and evacuation of blood clots and electrical cauterization. Refractory LHC such as the above situation or those who progress to

ADV viremia and disseminated disease were defined as severe LHC. Antiviral agent cidofovir were used for them.
Results: Overall, 33 patients (7.9%) developed severe LHC. The median day of presentation was 39 days (range 14 to 285days). LHC cases were graded as follows: grade 1 (3%), grade 2 (24%), grade 3 (64%), grade 4 (9%). Adenovirus viremia was detected in 18 patients (54%). The median duration of cidofovir treatment was 24 days (range, 3-54 days), and a median of 8 doses were given (range, 2-13 doses). A CR was recorded in 18 (54%) of 33 patients with severe LHC treated with intravenous cidofovir, whereas a PR was documented in 3 patients (9%). No improvement or worsening was observed in 12 (36%) patients, respectively. In total, 11 developed renal toxicity associated with cidofovir therapy, five developed bone marrow suppression. The overall mortality was 33% at 30 days and 67% at 180 days after initiation of cidofovir therapy, indicating that this was a very high-risk patient population.
Conclusion: We conclude that cidofovir may be a potentially effective therapy for severe LHC accociated with ADV and BKV, but the further prospective studies are required because of the very high mortality in severe LHC patients.
Disclosure of Interest: None declared.

P564
Impact of human herpesvirus 6 (HHV6) infection after pediatric allogeneic HSCT

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Introduction: Human herpes virus 6 (HHV6) has been increasingly recognized as a potentially life-threatening pathogen after after allogeneic hematopoietic stem cell transplantation (allo-HSCT). HHV6 infection/reactivation has been associated with delayed engraftment, myelosuppression, pneumonitis, encephalitis, and acute GVHD. However, its role on HSCT outcome is unclear, and data in the pediatric population are scarce.
Materials (or patients) and methods: We prospectively monitored blood HHV6 load weekly in the first month, and bi-weekly thereafter until day $+100$ by quantitative PCR in 170 pediatric recipients of allo-HSCT. Immune reconstitution, including virus-specific cellular immunity, was also monitored by flow cytometry and IFNγ ELISPOT analysis.
Results: At a median time of 19 (range 4-277) days posttransplant, 80 patients developed HHV6 viremia (cumulative incidence, CI 47%), that lasted 21 days. Median peak viraemia was 1025 copies/ml (range 50-7946900). Among the 80 viremic patients, 27 had a HHV6 viremia >3000 copies/ml (median peak 13400 copies/ml), with 8 showing severe virus-related disease.
HHV6 reactivation was more frequent in recipients of HSCT from partially matched family donors (CI: PMFD 63% vs MUD 54% vs MFD 22%; $P<0.001$), while in both matched family and unrelated transplants, there was no difference if the SC source was cord blood or bone marrow. T-cell depletion (TCD) was a risk factor for HHV6 reactivation, but TCD type did not impact on viral reactivation. We did observe a trend towards higher CI for ATG use (50% vs 28% in the non ATG group; $P<0.06$).
A status of T cell immune deficiency at the time of onset correlated with HHV6 reactivation, and a prompt recovery of virus-specific cellular immune function determined rapid resolution of viraemia.
In our cohort, HHV6 reactivation had no impact on neutrophil engraftment (HHV6-positive group: 18 days vs 19 days in HHV6-negative group), platelet engraftment (HHV6-positive group: 20 days vs 25 days in HHV6-negative group), grade II-IV acute GVHD occurrence (HHV6-positive group: 33% vs 31% in the HHV6-negative group), or overall survival (HHV6-positive group: OS 83% vs 76% in the HHV6-negative group).
Conclusion: Although the occurrence of HHV6 did not impact on survival after HSCT in our pediatric patients, severe HHV6-related disease that required antiviral therapy was observed. Therefore, HHV6 surveillance in the early post-HSCT period may be advisable in order to avoid treatment delay.
Disclosure of Interest: None declared.

P565
Increased incidence of CMV disease in patients with vitamin D deficiency before allogeneic stem cell transplantation

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Introduction: We have previously reported an association between vitamin D deficiency prior to allogeneic stem dell transplantation and an increased risk of chronic GvHD. Vitamin D deficiency has also been implied as a risk factor for infectious diseases. Several antimicrobial peptides, most notably LL-37 and beta-defensin 2, are dependent of vitamin D. These antimicrobial peptides have anti-bacterial as well as anti-viral activity.
Materials (or patients) and methods: This study aimed to evaluate possible associations of vitamin D deficiency prior to transplantation with infectious complications after ASCT. It is a retrospective cohort analysis on 137 patients who have undergone ASCT at Karolinska University Hospital, Huddinge, between 2005 and 2011. Children under the age of 12, cord blood transplantations and patients with follow-up at other hospitals than Karolinska were excluded. Data was collected from patient files. Vitamin D was analysed as 25-OH-cholecalciferol from cryopreserved serum samples using a chemiluminescence method (CLIA), by the laboratory for clinical chemistry at Karolinska University Hospital, Solna.
The outcomes studied were both agent-specific and non-specific. Agent-specific outcomes were CMV disease, EBV-associated PTLD, influenza, invasive fungal infections, and bacteraemia. Non-agent-specific outcomes were pneumonia and days on intravenous (iv) antibiotics. Bacteraemia and days on iv antibiotics were analysed separately for neutropenic and non-neutropenic episodes.
Results: Median level of vitamin D before transplantation was 39 nmol/L (range 10-118), hence below the level of insufficiency (50 nmol/L).
On individual analysis of each outcome, we found that vitamin D level prior to transplantation showed a statistically significant correlation to CMV disease ($P=0.005$) and to days on iv antibiotics during the non-neutropenic period ($P=0.011$). After a Bonferroni correction to account for multiple comparisons, only CMV disease remains statistically significant.
For CMV disease, we excluded patients who were CMV seronegative and received a CMV seronegative transplant ($n=15$). There were a total of 9 confirmed cases (incidence 6,6%) of CMV disease in the cohort, as defined by Ljungman et al. They comprised of 1 retinitis, 4 colitis, 1 gastric and 3 with involvement of multiple organs including the lung. All of the cases had vitamin D level prior to transplantation below the level of insufficiency (50 nmol/L) and 4 were below the deficiency level (25 nmol/L). The correlation between vitamin D level and CMV disease remained statistically significant after adjusting for patient age, CMV serologic mismatch, use of ATG, acute GvHD, chronic GvHD and graft failure in a multivariate Cox proportional hazards model, with death and relapse as competing risks ($P=0.002$). We then evaluated CMV viral load and viral replication kinetics with regard to vitamin D level, and found no association between vitamin D level and peak viral load, repeated or prolonged CMV reactivations, or viral replication kinetics.

Conclusion: To summarize, we found a statistically significant association between vitamin D level before HSCT and incidence of CMV disease in the first year. Vitamin D levels were not associated with invasive fungal infections, pneumonia, influenza or bacteraemia. Further studies are needed to elucidate this association.
Disclosure of Interest: None declared.

P566
Stratification and selection of IFI Risk Factors according to survey Delphi Method

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Introduction: Morbidity and mortality from invasive fungal infection (IFI) remains high in oncohaematologic patients and above all in allogeneic stem cells transplant (ASCT) recipients. In recent years, risk factors for IFI related to comorbidities, immunosuppressive therapy, the level of air pollution and others have been identified. However, there is still a need to categorize risk factors for IFI in order to improve the management of these patients.
Objective: The purpose of this study was to identify and categorize key risk factors for the development of a filamentous IFI in oncohaematologic patients and specifically in patients with ASCT (with or whithout Graft versus host disease [GVHD]).
Materials (or patients) and methods: When all the risk factors had been identified through literature review, a national prospective survey was conducted in Spain in order to reach consensus by the Delphi Method in November 2014. The study was carried out anonymously by e-mail contacting experts in hematology. Key risk factors were considered when a ''maximum'' or ''high'' answer was obtained for at least 70% of the experts.
Results: The panel was formed by 42 experts hematologist agreed to participate on the survey, and of these 28 (66.7%) completed the questionnaire. In the ASCT a 100% agreement was obtained for the presence of profound (<100) or prolonged (>14 d) neutropenia, prior IFI, GVHD III-IV or extensive chronic GVHD, both under inmunosupressors and corticoids. In the 99%>90% consensus was obtained for umbilical cord ASCT, allogeneic mismatched HLA and failure of the graft. For a previous diagnostic of acute myeloid leukemia, an haploidentic HSCT, administration of anti-TNF or alemtuzumab for specific haematological diagnoses, the agreement was in the range of 89-80%. Proximity to areas under renovation or in building works, rooms without HEPA filters or laminar flow also achieved the consensus of 89-80% of the experts.

AGREEMENT RATE (Maximum & High Risk)	*KEY RISK FACTORS FOR ALLOGENEIC HSCT RECIPIENTS*
100%	- Deep (<100) or prolong (>14 d) neutropenia - Prior IFI - GVHD III-IV under inmunosupressors and corticoids - Extensive chronic GVHD under inmunosupressors and corticoids
99%–90%	- Umbilical cord HSCT - Allogeneic mismatched HLA - Failure of the graft
89%–80%	- Previous diagnostic of acute myeloid leukemia, - Haploidentic HSCT - Administration of anti-TNF α - Administration of alemtuzumab - Proximity to areas under construction, - Rooms without HEPA filters - Rooms without laminar flow
79%–70%	- Lymphocitopenia (<200) - Prophylaxis with inmunosupressors for GVHD - Antithymocyte globulin

Conclusion: The Delphi method has proved to be a useful tool to establish and categorize key risk factors for the ASCT patients and others with hematological cancer. Key risk factors can help in the management of these patients at risk of suffering IFI, in order to decide an adjustment of prophylaxis or starting early antifungal treatment.
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P567
Efficacy and Cost-effectiveness of a different schedule for the prevention of fungal infections during the early phase of stem cell transplantation

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Introduction: Antifungal prophylaxis (AF) during neutropenia following SCT is recommended, especially in allo-SCT, however it is associated with adverse events, drug interactions or high prices. Except when needed secondary AF, we use environmental protection (HEPA air and water filters) and only start antifungal treatment at the 1st peak of fever during the neutropenic phase of autologous (auto-SCT) or allogeneic (allo-SCT) transplant. We evaluate retrospectively the incidence of fungal infections (IFI) during the early phase and compare the real cost of our procedure with the hypothetical cost if we had used posaconazole (200 mg/8 h po), voriconazole (200 mg/12 h iv) or micafungin (100 mg/24 h iv), started at the beginning of conditioning or started the infusion day.
Materials (or patients) and methods: 215 patients allo-SCT (88 MRD, 67 MUD, 60 MmD) and 130 auto-SCT were evaluated. Median age was 49 years (allo-SCT) and 58 years (auto-SCT). The main underlying disease was acute leukemia/MDS (133). BM in 158 patients was the main stem cell source in allo-SCT. Efficacy was assesed evaluating the development of probable or proven IFI according with the EORTC-2008 criteria during the first 60 days post-SCT, the type of microorganism, the cause of death and autopsy diagnosis. We compared the efficacy, with that reported in the literature. To assess the cost of our procedure (Group V1), we evaluated the days on the protected environment room, the days of hospitalization and the type and days of antifungal tretment. The hypothetical cost of the standard prophylaxis was calculated in each patient based on the beginning of the conditioning regimen (Groups CPos, CVor, CMic) or the day of infusion (Groups TPos, TVor and TMic) to outpatient. Cost/day of HEPA and water filters was 3.4 €. Drug costs were calculated based on our local prices. Differences were calculated with the paired two-sample t-test.
Results: Two proven (*C.parapsilosis* and *Alternaria* sp.) and 3 probable (2 *Aspergillus* and 1 *Mucor*) IFI were diagnosed during the early phase (incidence 1.45%). At day +60, 16 patients had died, 2 of them because of fungal infection (1 *Aspergillus* and 1 *Mucor*). Another fungal infection was detected in 1 of the 8 autopsies made (Cumulative incidence 1.5%). Median duration of conditioning regimen, hospitalization and isoation in the protrected room was 6 days, 25 days and 24 days respectively. 47 patients (14%) did not need any treatment. Patients were treated only with fluconazole (91) or with an echinocandin (59) and 68 patients with fluconazole followed by echinocandin. The mean cost of Group V1 was 1851 €, statistically different ($P < 0.001$) when it was compared with Groups CVor (7936 €), TVor (6400€), CMic (10974 €) and TMic (8850 €). We did not find differences in costs when the Group V1 was compared with posaconazole (CPos 2666 € and TPos 2150 €, $P = 0.066$ and $P = 0.973$ respectively).
Conclusion: We present a different schedule for the prevention of IFI during the neutropenic period of SCT with the same efficacy as reported by Cornely (2007), Wingard (2010), Van Burik (2004), and lower cost than voriconazole or micafungin

in primary prohylaxis. Although we did not observe differences with the posaconazole group, we did not include in the analysis several factors (mucositis, hepatic impairment, fungal suspiction) that can modify its continued use.
Disclosure of Interest: L. Yañez Conflict with: Gilead, MSD and Pfizer Speakeŕs bureau. Gilead, MSD, Pfizer and Astellas advisory m., N. Fernandez: None declared, A. Bermudez: None declared, A. Insunza: None declared, C. Richard: None declared, E. Conde: None declared.

P568
Improving the Diagnostic Algorithm for Sepsis: Adjuvant Role of SeptiFast in 491 Consecutive Hematological Patients

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Introduction: Febrile neutropenia and sepsis are frequent and life-threatening complications in patients with hematological malignancies. Although the proportion of infectious deaths in high-risk patients has decreased over the last two decades, much remains to be done to further reduce these events. Blood cultures (BC) identify a pathogen in only 20 to 30% of febrile episodes, the culturing and pathogen identification process is lengthy, postponing the start of a pathogen-targeted treatment. Thereby, a sensitive tool to promptly recognize pathogens causing sepsis is of high clinical relevance.
Materials (or patients) and methods: We assessed the diagnostic usefulness of the LightCycler Septi*Fast* test (SF; Roche Molecular Systems), a PCR-based multiplex assay performed on peripheral blood and capable of detecting 25 among the most common species isolated in sepsis. The assay uses dual fluorescent resonance energy transfer (FRET) probes against the species-specific internal transcribed spacer (ITS) regions, a non-coding sequence interspaced among highly conserved bacterial and fungal RNA. Time from processing to result is remarkably short (less than 6 hours). In this study, blood samples from febrile haematological patients were concomitantly tested by traditional blood culture (BacT/Alert 3D; bioMerieux).
Results: A total of 1837 blood samples were collected from 491 consecutive hematological patients treated for febrile neutropenia at the San Raffaele Hematology and Bone Marrow Transplantation Unit, from 2009 to 2013. Out of the total 1837 episodes examined, positive results were detected in 520 samples by SF (28%), and in 318 by BC (17%). Together, the two methods identified a total of 742 microorganisms in 664 (36%) episodes: Gram-positive bacterial species (77%), Gram-negative bacterial species (21%), and fungal species (2%). Concordance between the two methods was 74%, with most of the discordant samples that tested negative by culture but positive using the molecular approach (78% of the total positive samples). The cases positive by SF alone were mostly samples from patients already receiving antimicrobial therapy (76%), persistent fever (63%) or non-bacteremic infections (66%); importantly, four samples were positive for fungal pathogens such as *Aspergillus fumigatus*, which is hard to detect by the traditional approaches. Mainly gram-positive bacteria (60% coagulase-negative staphylococcal pathogens) were recorded in the cases positive only by BC, suggesting a possible adjuvant role of molecular tools to discriminate a sample contamination from the isolation of pathogens causing sepsis.
Conclusion: This large analysis demonstrates a significant correlation between the molecular test and the standard BC in hematological patients with febrile neutropenia and sepsis. Septi*Fast* may be included as a molecular diagnostic tools in the traditional diagnostic algorithm of sepsis, particularly in persistent fever despite antibacterial therapy, when a non-responding bacterial infection or an invasive fungal infection is suspected, therefore leading to a rapid diagnosis and an earlier targeted antimicrobial therapy. Although promising, this molecular test is still not developed for the identification of drug resistance markers, crucial for the emerging MDR gram-negative bacteria; new microarray tests are under investigation in this peculiar setting.
Disclosure of Interest: None declared.

P569
The influence of lymphocyte subsets evaluated before autologous peripheral stem cell transplantation on severe infectious complications in patients with multiple myeloma

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Introduction: Autologous peripheral blood stem cell transplantation (APBSCT) is now established therapy for patients (pts) with multiple myeloma (MM). Despite of clinical efficacy, this method may be associated with profound haematopoietic and immune suppression resulting in infectious complications. In this study we analyzed the influence of lymphocyte subsets evaluated before APBSCT in pts with MM on the incidence of severe infections.
Materials (or patients) and methods: A total of 50 (27 male and 23 female; median age 58.0) consecutive pts with MM at the Department of Haematooncology and Bone Marrow Transplantation at the Medical University of Lublin were recruited to this study. The day before myeloablative regimen samples for flow cytometry analysis were taken. In the transplantation procedure pts were received melphalan (140 or 200 mg/m^2) followed by infusion of haematopoietic cells (median number 4.5x10^6/kg; 2.2-9.0). Pts received standard prophylaxis of infection (ciprofloxacin, fluconazole, aciclovir) and G-CSF. The incidence of severe infectious complications (sepsis) was referred to lymphocyte subset number before AHSCT.
Results: The median days to ANC $>0.5 \times 10$^9/L recovery was 13.0 days (10-48). Five pts developed sepsis in neutropenic phase. The etiology of infections was following: *Stahyloccoccus eipdermidis* in 1 pt, *Staphylococcus aureus* in 1 pt, *Klebsiella pneumoniae* in 1 pt *ad Escherichia coli* in 2 pts. There was no case of death in any pts. Analysis of flow cytometry results showed in these 5 pts reduced number (with statistical difference compared to pts without sepsis and control group) of following subsets of lymphocytes: helper T cells (CD3+ CD4+ CD45RA+; $P=0.005$), lymphoid dendritic cells (BDCA2+ CD123+; $P=0.02$) and NKT-like cells (CD3+ CDD56+; $P=0.04$). There was no statistical relationship between sepsis incidence and other analyzed lymphocyte subsets (B cells, suppressor T cells, myeloid dendritic cells, NK cells ad regulatory T cells).
Conclusion: Analysis of lymphocyte subsets before AHSCT may be useful for defining of pts group with high risk of severe infection. In this selected group special antibiotic therapy and infusion of higher number of mononuclear cells may be helpful in prophylaxis of infection.
Disclosure of Interest: None declared.

P570
PICC-related complications in autologous hematopoietic stem cell transplant recipients

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Introduction: Central lines are an important part of the support treatment for hematopoietic stem cell transplants

recipients. During the last few years the use of peripherally inserted central catheters (PICC) has been implemented with excellent outcomes.
In our service the PICC lines are the preferred venous access due to celerity of insertion and lower percentage of complications related to catheter. All patients have the venous sites integrity assessed by ultrasound previous insertion. Limitations of the technique are mostly due to difficult venous access which can limit the availability of it to every patient.
Our objective was to analyze the PICC-related complications in our service.
Materials (or patients) and methods: Retrospective analysis of 31 adult patients of the Fundación Jimenez Díaz Hematology Service, who had a PICC line for their autologous stem cell transplant, between January 1st 2012 and December 15th 2014 was made.
The insertion technique used by our Venopuncture Team was the Modified Seldinger technique, in which the catheter is inserted in an aseptic environment, guided by ultrasound and measuring vein calibers, and using a micropunction needle to minimize the damage done to the endothelium.
The median age was 54 years old (range: 20-68). The transplant indication were in 65% multiple myeloma, 29% refractory lymphomas and 6% for other diseases.
93% of the catheters were placed on the upper right extremity, with two lumens and a maximum diameter of 5 french.
None of the patients had a previous history of thrombosis or catheter-related infections. No systemic thromboprophylaxis was used. All patients followed the heparinisation protocol every seven days with one vial of 3 ml of sodium heparin (20 UI / ml).
Results: None of the 31 PICCs are being currently used at this time. The median removal time was 26 days, being the main reason for it in 88% wasn't needed anymore, death in 6%, thrombosis in 3%, and other motives in 3%. None of the catheters were removed due to infection.
The catheter-related thrombosis was clinically and ecographic confirmed in the right basilica vein and extending to the right subclavian vein. Patient was started on low weight heparin but after having fever we suspected infection and the catheter was removed.
Conclusion: In our series we describe a 3% thrombosis rate, which is a low percentage considering the high-risk features of our patients.
Furthermore there weren't any catheter-related infections, after removal all the catheters were cultured with all being negative. Team specialized in ultrasound PICC guided insertions has diminished the incidence of catheter complications in our series.
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Disclosure of Interest: None declared.

P571

Risk factors associated with Pneumocysctis jirovecii pneumonia after allogeneic stem cell transplantation; when can PCP prophylaxis be discontinued?

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Introduction: *Pneumocystis jirovecii* pneumonia (PCP) has become a rare opportunistic infection in allogeneic stem cell transplant (alloSCT) recipients due to effective prophylaxis. However, several studies have reported on late-onset PCP after discontinuation of prophylaxis, and there are no clear definitions of parameters that are sufficient to discontinue PCP prophylaxis in alloSCT recipients. To investigate possible parameters, we undertook the retrospective study of PCP after alloSCT in our hospital.
Materials (or patients) and methods: We retrospectively analyzed 955 alloSCT recipients who received first alloSCT from January 2004 to December 2013 in Toranomon Hospital. The diagnosis of PCP was made by clinical symptomssuch as fever, cough and progressive dyspnea, bilateral infiltrates on chest radiography or thoracic computed tomography, elevated levels of 1,3-beta-D-glucan in serum, and detection of *Pneumocystis jirovecii* in respiratory specimens by PCR or Grocott staining. Broncho-alveolar-lavage fluid was mainly used as respiratory specimens. Oral trimethoprim-sulfamethoxazole (TMP/SMX) was used as first choice of prophylaxis for PCP, and aerosolized pentamidine or oral sulfadiazine/pyrimethamine was alternatively used for those TMP/SMX was intolerable. Onset of PCP less than 100 days after alloSCT was defined as early-onset, and that 100 days and later after alloSCT was as late-onset.
Results: Six hundred and five (63%) patients were male, 663 patients (69%) were in high risk disease status, and myeloablative conditioning (MAC) regimens were selected in 441 patients (46%). Related PBSCT/BMT ($n = 145$), unrelated BMT ($n = 220$), and unrelated CBT ($n = 590$) were included. Six hundred and fourteen patients (64%) survived 100 days and more after alloSCT. Eleven patients developed PCP after alloSCT (early-onset PCP in 2 patients and late-onset in 9 patients), and cumulative incidence of PCP was 2.2% (rBMT/PBSCT 0%, uBMT 1.0%, and uCBT 3.7%, $P = .201$). All but one patient with PCP were cured by treatment with TMP/SMX and corticosteroids. PCP did not develop in any patients under continuing prophylaxis. The median duration from alloSCT to onset of late-onset PCP was 692 days (range 255-2617). All patients with late-onset PCP had chronic graft-versus-host diseases (cGvHD), had more than 200/uL of $CD4^+$ T cells (median 608/uL, range 213-2038), and developed PCP at less than 5 months after discontinuation of PCP prophylaxis (median 83 days, range 39-144). The use of MAC regimens was identified as a risk factor of PCP by multivariate analysis. For those with late-onset PCP, presence of cGvHD as well as the use of MAC regimens was identified as risk factors. The incidence of late-onset PCP was higher in CBT group than in the other in univariate analysis (5.7% vs. 0.4%, $P = .02$), but it was not statistically significant in multivariate analysis ($P = .08$). The incidence of late-onset PCP in patients who stopped PCP prophylaxis in less than 12 months after discontinuation of immunosuppressants was as same as in those who later than 12 months (4.1% vs. 3.7%, $P = .368$).
Conclusion: Late-onset PCP could develop in alloSCT recipients who had $CD4^+$ T cells greater than 200/uL, or in those who had discontinued immunosuppressants for longer than 1 year. No specific parameters that indicate optimal timing of discontinuing PCP prophylaxis could be identified from this analysis.
Disclosure of Interest: None declared.

P572

Association of low counts of CD16 + /14- monocytes with CMV infection after hematopoietic stem cell transplantation

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Introduction: CMV infection is a common complication after hematopoietic stem cell transplantation (HSCT), usually leading to pancytopenia and increased hospitalization time. CMV infects macrophages impairing their function, evading host defenses and impairing their function in vitro. CD16 + /14- monocytes (16 + Mo) are differentiated monocytes witch presents features of dendritic cells (DCs) as high antigen uptake and presentation, and are considered to be circulating macrophages. On the other hand myeloid (mDCs) and plasmacytoid DCs (pDCs) have higher resistance to infection and drives host defenses against the virus. Our objetive was to investigate CMV infection and DC and 16 + Mo populations after HSCT.
Materials (or patients) and methods: DCs [lineage negative, HLA-DR + +: 123 + (pDC) or 11c + (mDC)] and 16 + MO (16 +, 14-, HLA-DR + +) were quantified by multiparametric flow cytometry at 9 sequential time points (before conditioning, at engraftment, and at days 3, 7, 14, 21, 60, 100 and 180 after engraftment). Overall, 111 patients, from 4 HSCT centers (65% male, median age 17 years, range 1-74), receiving bone marrow (BM, 46%), umbilical cord (UCB, 32%) or peripheral blood (PB, 22%) from unrelated ($n = 90$) or related donors ($n = 21$) were studied. The most common diagnosis was acute leukemia (AML 36%, ALL 31%, MDS 9%, CML 9%, Aplastic anemia 8%). Most patients received myeloablative conditioning (MAC) regimens (60%). Antithymocyte globulin (ATG) was used in 44 patients 40%) and total body irradiation (TBI) in 56 (51%). Median follow up time was 21 months (range 4-48). CMV was monitored twice a week by RT-PCR in the first 100 days.
Results: 86 patients presented sustained allogeneic recovery (no differences among sources) and median time to neutrophil engraftment was 18 days (range: 8-52). Forty-two patients presented CMV reactivation (CMV +)(median time to first reactivation = 37 days, range = 11-67; four patients had first reactivation after 1 year). Ten patients had CMV symptomatic disease, all before D + 100. CMV infection did not affect mDC, pDC, TCD4 and TCD8 populations' recovery. However, CMV + patients presented significant lower counts of 16 + MO after engraftment then CMV- patients at day 14 (CMV + vs. CMV-; median: 30 cells/μL vs. 73 cells/μL, $P = 0.042$), day 21 (22 vs. 59 cells/μL, $P = 0.003$) and day 60 (20 vs. 51 cells/μL, $P = 0.006$). These patients had the same monocyte counts as CMV- patients in all time points, and flow cytometry analyses show a blockage of monocytes maturation into 16 + MO. There was no significant influence of conditioning in DCs and 16 + MO cell counts.
Conclusion: Patients who reactivated CMV after transplant showed impaired 16 + MO recovery after transplantation with normal monocyte counts. This may represent CMV-induced impairment of monocyte differentiation *in vivo*, suggesting a viral mechanism of immunomodulation and evasion from host defenses. Further studies are necessary to confirm this hypothesis.
Disclosure of Interest: None declared.

P573

Impact of single or associated CMV, EBV and BK virus reactivation early after allogeneic hematopoietic stem cell transplantation on relapse incidence

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Introduction: Many recent studies have evaluated the impact of cytomegalovirus (CMV) reactivation after allogeneic hematopoietic stem cell transplantation (allo-HSCT) showing a significant association with reduced risk of relapse. On the other hand, other frequent viral infections or reactivations like Epstein-Barr virus (EBV) and BK virus (BK-V) have not been evaluated in the same context. The aim of this study is to evaluate the impact of CMV, EBV and BK-V reactivation up to 3 months after allo-HSCT whether alone or associated on the relapse incidence of patients with hematological malignancies.
Materials (or patients) and methods: We evaluated 359 consecutive patients with hematological malignancies who received allo-HSCT and were followed in our center between January 2008 and June 2013; there were 218 (61%) males and 141 (39%) females with a median age of 48 years (range: 18-70), 182 (51%) had AML, 44 (12%) multiple myeloma, 34 (9%) MDS, 30 (8%) NHL, 7 (2%) CLL, 21 (6%) MPS, 14 (4%) Hodgkin disease, 13 (4%) CML and 14 (4%) aplastic anemia. At transplantation, 227 (63%) patients were in complete response (CR) or chronic phase (CP) and 132 (37%) were in less than CR or CP. For conditioning regimen, 171 (48%) were myeloablative and 188 (52%) were reduced intensity. DNA levels of CMV, EBV and BK-V in blood were detected by quantitative real-time polymerase chain reaction (RQ-PCR) after weekly monitoring up to 3 months after allo-HSCT. CMV-DNA, EBV-DNA or BK-V-DNA was considered positive when the copies exceeded 1000 copies/ml.
Results: Among 359 patients, there were 102 patients who had CMV reactivation after a median time of 1.4 months (1.1-1.8) after allo-HSCT with a cumulative incidence of 25% (24-26) at 3 months; 222 patients had EBV reactivation after a median time of 1.3 months (0.7-2.5) after allo-HSCT with a cumulative incidence of 48% (47-50) at 3 months; and 38 patients had BK-V reactivation after a median time of 1.1 months (0.7-1.5) after allo-HSCT with a cumulative incidence of 10% (9-11) at 3 months. The cumulative incidence of relapse at one and two years for the whole population was 27% (26-28) and 34% (33-35) respectively and the cumulative incidence of transplant-related mortality (TRM) was 22% (21-23) and 25% (24-26) respectively. The multivariate analysis taking into account the type of disease, the type of conditioning, the disease status at transplantation, the presence of acute GVHD and single or the association of viral reactivation. This analysis showed that the presence of a single viral reactivation was associated with a significant lower relapse rate, for CMV: sdHR = 0.34 [0.12-0.92], $P = 0.03$, for EBV: sdHR = 0.52 [0.35-1], $P = 0.05$ and for BK-V: sdHR = 0.58[0.24-0.7], $P = 0.002$; and that patients who have an associated CMV and EBV reactivation had significantly higher risk of relapse, sdHR = 5 [1.59-16], $P = 0.006$. There was no significant impact of these reactivations on TRM.
Conclusion: We confirmed the positive impact of CMV reactivation on relapse incidence, in addition we demonstrated that this impact exists also for EBV and BK-V, however we showed for the first time that the association of CMV and EBV was significantly associated with a higher risk of relapse. More investigations are ongoing to evaluate the immunological status of these patients and the different administered anti-viral treatments.
Disclosure of Interest: None declared.

P574

Risk factors and clinical outcome for Herpes Simplex Virus reactivation in patients after allogeneic hematopoietic stem cell transplantation

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Introduction: Immune reconstitution is often impaired for months to years in patients after allogeneic hematopoietic stem cell transplantation (HSCT) which predisposes them to a wide collection of clinical infections.
Primary herpes simplex virus (HSV) types 1 and 2 infections in immune-competent hosts results in long-term latent infections that can be associated with recurrent reactivations. After HSCT symptomatic HSV-1 reactivation predominantly manifests with involvement of the oropharyngeal, esophageal or tracheobronchial tracts.

Without prophylaxis HSV disease after HSCT occurs in approximately 70–80% of seropositive recipients. Although it is rarely life-threatening, HSV reactivation may cause severe oral and genital ulceration and in some cases even disseminated diseases such as encephalitis. Our center follows the current guidelines that recommend antiviral prophylaxis for HSV-seropositive allogeneic HSCT recipients against HSV infection which has reduced the incidence of HSV disease in the early phase after HSCT.

Materials (or patients) and methods: We retrospectively analyzed possible risk factors both by univariate and multivariate analysis for HSV reactivation in 382 HSV seropositive patients transplanted between 2005 and 2013 at our center. In addition we analyzed the impact of HSV reactivation on clinical outcome in this patient cohort with regards to; overall survival (OS), relapse free survival (RFS) and Transplant related mortality (TRM).

Results: The cumulative incidence was 18% of early HSV reactivation (<90 days) in the patient material. A majority (67%) of the HSV infections occurred within 30 days after HSCT. HSV infection occurred a median of 20 days (5-90) after HSCT. The only risk factor observed for HSV reactivation both by univariate and multivariate analysis was age of recipient ($P<0.01$). The cumulative incidence of HSV reactivation in the different age subsets were: 0-20 years: 12.5%, 21-40 years 14.9%, 41-50 years 16.9%, 51-60 years 22.9% and >60 years 24.6%. There was no significant difference in OS, RFS or TRM in the patient material with regards to HSV reactivation. However, patients with malignant disease patients with HSV reactivation had significantly decreased OS compared to patients with no HSV reactivation ($P=0.02$). In patients with lymphoma this difference was even more prominent ($P=0.002$). There was also a significant decrease in RFS in this patient category ($P<0.01$).

Conclusion: Despite antiviral prophylaxis there is still a substantial proportion of HSV reactivation in patients after HSCT. In our material we did not observe any risk factors except older patient age. While the OS and RFS was significantly decreased in lymphoma patients with HSV reactivation additional measures as intensified prophylaxis and additional therapy might be considered. Future studies regarding the mechanism of HSV reactivation and lymphoid malignancies are warranted.

Disclosure of Interest: None declared.

P575

Drug interaction and safety profiles of concomitant use of caspofungin and tacrolimus in allogeneic hematopoietic stem cell transplant recipients

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Introduction: Invasive fungal disease is one of the life-threatening complications of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although a variety of antifungal agents are needed for the clinical management of hematological disorders, their interactions with calcineurin inhibitors is a problem requiring elucidation. A Phase I study in healthy subjects reported that caspofungin (CPFG) could potentially reduce tacrolimus (TAC) concentrations by up to 20%.Although the only two case series data available for solid organ transplant did not reveal anything significant in their safety and drug interaction profiles, nothing is known of this regard in allo-HSCT settings.

Therefore, we retrospectively assessed the drug interaction and safety profiles of allo-HSCT recipients treated concomitantly with CPFG and TAC at our institution.

Materials (or patients) and methods: Following allo-HSCT performed at our institution, we investigated those patients who received concomitant therapy with CPFG and TAC between May 2012 and October 2014. All patients who had received concurrent treatment with both CPFG and TAC for at least 1 day were included in this study. Of those, we also evaluated drug interactions in patients who had already been on a steady dose of continuous intravenous TAC before initiating CPFG. To assess hepatotoxicity, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used. Serum creatinine (Cre) was used to assess nephrotoxicity. The serum concentration/dose (C/D)-ratio of TAC was calculated by dividing the measured serum concentration of TAC (ng/ml) by the total daily dose (mg/kg) the patient was receiving at the time. The C/D-ratio was assessed just before and 5-7 days after the initiation of concomitant therapy. We compared the changes in C/D-ratio between before and after the initiation of concomitant therapy.

Results: A total of consecutive 36 cases, median age 51 (23-67) years, were eligible for inclusion in this study and received concomitant therapy for 3–83 days (median 12.5). Of those, C/D-ratio data were determined in 13 cases. In these patients, the median duration of concomitant therapy was 13 days (5–31) and there were no statistically significant differences in C/D ratio between before and after the initiation of concomitant therapy (597.6 with a range of 237.6-1265.8 and 680.2 with a range of 195.3-1016.1, respectively; Wilcoxon signed-rank test. $P=0.50$). In analyses on the hepatotoxicity and nephrotoxicity in 36 cases, there were no statistically significant changes in AST, ALT and Cre values before and after co-administration (Wilcoxon signed-rank test. $P=0.67$, $P=0.64$ and $P=0.87$, respectively). There were no patients who discontinued CPFG due to the adverse effects of concomitant use.

Conclusion: Our data showed that drug interactions between CPFG and TAC were of negligible clinical significance. As oral TAC was used in the Phase I study, the occurrence of drug interactions may have been minimized by the use of intravenous administration. Alternatively, there may be ethnic differences in drug metabolism including CYP. In addition, concomitant use did not significantly affect either liver or kidney function in allo-HSCT recipients. In the future, a larger, adequately powered study will be required to confirm our results.

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P576

Enumeration of CMV-specific CD8 and CD4 T-cells with HLA-tetramers after first CMV reactivation identifies SCT patients at risk of recurrent reactivation, and provides a stratified approach to secondary prevention of recurrent viraemia

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Introduction: CMV reactivation remains a major cause of morbidity following T-cell depleted allogeneic stem cell transplantation (HSCT) in CMV-seropositive patients. In this high risk group the CMV reactivation rate approaches 85%, and as many as 50% of these patients develop recurrent viremia with significant post-transplant complications. Currently there is no established method for predicting CMV recurrence in HSCT. Both CMV-specific CD8+ and CD4+ T-cells have been shown to be important in maintaining viral control, though the role of CD4+

T-cells has been less clear, due the difficulty in directly identifying CMV-specific CD4+ T-cells.
Materials (or patients) and methods: CMV-specific HLA-class I and novel HLA-DR7 tetramers were used to monitor the global CMV-specific T-cell reconstitution post-HSCT in patients at high risk of CMV reactivation. CMV-specific CD8+ T-cells were enumerated in 27 patients, and CMV-specific CD4+ T-cells in 20 patients. The results were correlated with viral control to assess the utility of assaying CMV-specific T-cell levels in clinical practice.
Results: CMV-specific CD8+ and CD4+T-cell levels were very low or undetectable in patients early after transplant, but expanded in parallel in response to the first viral reactivation ($r = 0.75$, $P < 0.0001$). The number of CMV-specific CD8+ and CD4+ T-cells after the first viremic episode was significantly higher in patients with a single viremic episode compared to those who experienced recurrent episodes ($P = 0.007$ for CD8; $P = 0.02$ for CD4).
Levels less than 20 x 10^3/ml CMV-specific CD8+ T-cells, and less than 0.7x10^3/ml CMV-specific $CD4^+$ T-cells after the first reactivation post-transplant, but not at baseline were predictive of viremic recurrence. CD57 was found to be a useful surrogate marker for total CMV-specific $CD4^+$ T-cells.
Conclusion: Low CMV-specific CD8+ and CD4+ T-cell levels after clearance of first CMV reactivation identify patients at high risk of recurrent viremia. HLA-tetramers allow risk-stratification of patients for secondary prophylactic or pre-emptive therapy with either second line antiviral drugs or adoptive immunotherapy with CMV-specific CTL.
Disclosure of Interest: None declared.

P577
Cytomegalovirus Infection in Children after Bone Marrow Transplantation: Risk factors, Clinical aspects and Outcomes

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Introduction: Cytomegalovirus (CMV) infection remains the most common and potentially severe viral complication in patients given HSCT. The aim of this single center retrospective study to determine the incidence, risk factors, clinical aspects and outcomes of CMV infection in our pediatric BMT unit.
Materials (or patients) and methods: This study was a retrospective analysis of clinical, laboratory and outcome data of all pediatric patients underwent BMT, at King Faisal Specialist Hospital and Research Centre (KFSH&RC)-Jeddah, Saudi Arabia, from July 2005 to June 2014. Data was stratified in two groups (CMV positive and CMV negative) to identify the risk factors associated with the development of CMV in post BMT patients and their outcomes. A *P*-value of < 0.05 was considered statistically significant.
Results: During the study period 95 pediatric patients were admitted for BMT. The mean age of the study population at the time of transplant was 6.5 ± 4 years. Out of males were 63 (66.3%) and females were 32 (33.7%). Majority of patients have hematological malignancy ($n = 31$; 32.6%); out of them ALL ($n = 9$), AML ($n = 15$), lymphoma ($n = 7$), followed by non-malignant disorder ($n = 30$, 31.5%) including beta-thalassemia ($n = 13$), sickel cell anemia ($n = 8$), aplastic anemia ($n = 6$), fanconi anemia ($n = 3$), solid tumors ($n = 19$, 20%) including neuroblastoma ($n = 16$) and medulloblastoma ($n = 3$) and HLH ($n = 5$; 5.3%) and others miscellaneous disorders ($n = 11$, 11.6%). Most of the patients received allogeneic transplant ($n = 71$; 74.5%) and remaining were autologous transplant ($n = 24$; 25.7%).
CMV reactivation was observed in 29 patients (29/95; 30.5%) within 100 day of post BMT. Out of them majority were asymptomatic ($n = 21$; 77.8) and remaining ($n = 9$; 22.2%) had clinical manifestation/organ involvement (Liver, Skin, GIT and CNS).
Age less than 5 year ($P = 0.043$), AML ($P = 0.019$), patients with positive pre-transplant CMV status ($P = 0.007$), conditioning regimen containing ATG ($P < 0.041$), allogeneic BMT ($P < 0.027$) lymphopenia $< 300/mm^3$ ($P = 0.049$) were identified as risk factors associated with development CMV reactivation in post BMT pediatric patients. A total of 36 (37.9%) patients developed GVHD and overall 27 (28.4%) patients were expired. Both outcome variables were statistically significant GVHD (OR: 5.4; 95% CI: 2.42-12.18) and mortality rate (OR: 8.1; 95% CI: 2.51-25.61) in patients with CMV reactivation versus no CMV reactivation respectively.
Conclusion: Young age, AML, positive pre-transplant CMV status, ATG containing conditioning regimen, allogeneic BMT and lymphopenia were identifiable factors associated with development CMV reactivation in post BMT pediatric patients.
Disclosure of Interest: None declared.

P578
A Study to Validate the Rationale and Efficacy of a Pre-emptive approach to the management of Cytomegalovirus (CMV) reactivation post allogeneic haematopoietic stem cell transplantation (HSCT)

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Introduction: Pre-emptive therapy for CMV reactivation post allogeneic haematopoietic stem cell transplantation (HSCT) has improved patient outcomes. At our institution, pre-emptive antiviral therapy is initiated when the viral load reaches the level of 1000 copies, (or > 500 on 2 consecutive occasions in the volunteer unrelated transplants), which were chosen based on a literature review and local knowledge of the sensitivity of the test. Here, we investigated the efficacy of our protocol and the kinetics of the response to antiviral therapy.
Materials (or patients) and methods: We conducted a retrospective analysis of all the medical records and CMV PCR data of all the HSCT carried out at our institution between Jan 2004 and July 2011.
Results: A total of 263 HSCT were carried out, and 184 of these were either Recipient (R) or Donor (D) CMV seropositive prior to transplant, and so were at risk of CMV reactivation. Median age was 44 years (19-67). 79 patients were myeloablative transplants vs 105 which were reduced intensity. Donor types were 88 matched sibling, 8 mismatched family member, 57 volunteer matched unrelated, 17 mismatched volunteer unrelated, 14 haploidentical transplants.
Of the 184, 87 patients reached our threshold for treatment and 37 patients had only low-level reactivations that did not reach threshold levels and were not treated.
Of these 87 patients

CMV serostatus	*R-D+*	*R+D+*	*R+D-*		
Transplant intensity	5 myeloablative	51 Reduced intensity	31		
Donor type	37 Sibling	50 Mismatched family	Matched unrelated	Mismatched unrelated	Haplo-indentical
	20	2	15	7	6

Medical records were available for 71 of 87 patients. Antivirals were started at a mean of 3.4 days post threshold (median 1.5 days). 63 of 71 patients responded to antiviral therapy. Of 13 patients who required 2nd line treatment, 7 responded whereas 6 died. All four patients who were not treated despite reaching threshold levels achieved pcr negativity.
Factors that influence efficacy of antiviral therapy
The threshold level viral load and the time from the threshold level to the initiation of antiviral therapy did not affect the

overall efficacy of the antivirals. Patients, who received haploidentical or mismatched family transplants took a median of 28 days to achieve negativity compared with 16 days for all other donor types ($P = 0.007$). T cell depleted transplants also took longer to achieve negativity compared to T replete transplants, 20 vs 15 days respectively, ($P = 0.027$). In multivariate analysis, donor type (RR = 2.93, $P = 0.016$), and T cell depletion (RR = 1.76, $P = 0.05$) are independently statistically significant. The risk of subsequent reactivation was significantly higher for a threshold level reactivation when compared to a low level reactivation (chi square 6.7781, $P = 0.009$).
Eight patients developed CMV disease and 4 responded to antiviral treatment. However, 5 of these 8 patients subsequently died within the context of a rising CMV PCR level, and radiological or histological evidence of CMV disease.
Conclusion: Pre-emptive antiviral strategy is effective in reducing death from CMV. Our data raise the questions about the threshold levels for treatment since time to antiviral treatment did not have much effect on overall efficacy. Our observations lay the foundation for the introduction of cellular therapy in the pre-emptive strategy.
Disclosure of Interest: None declared.

P579
Fatal measles myocarditis in a hematopoietic stem cell transplant recipient

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Introduction: Most patients lose immunity to measles after allogeneic hematopoietic stem cell transplantation (HSCT).
Materials (or patients) and methods: A 2.5 year-old Turkish girl with thalassemia major received allogeneic HSCT from her matched-sibling. Transplantation was successful and she had no GVHD. Immunosuppressive therapy was stopped on the post-transplant 3rd month. She had a history of measles mumps and rubella vaccine when she was 13 month-old.
On the post-transplant 14th month she presented with fever and rash. Mother reported that she refused food and liquids in the last 24 hours and had no urine. She had axillary temperature 38.1 °C, heart rate 145/min, respiratory rate 30/min, blood pressure 70/46 mmHg and SPO_2 98%. She seemed dehydrated and had agitation. Oscultation of the lungs and other physical examination findings were normal. Blood counts were WBC 6.4x10^9/L, ANC 4.8 x10^9/L, Hgb 10.8 g/dL, PLT 181 x10^9/L. She had hyponatremia and serum urea and transaminases were increased. She was monitorised and serum physiologic 20 ml/kg / hr was started. Cefepime 150 mg/kg/day was also started. On the third hour blood pressure increased gradually to normal limits (98/59 mmHg) and SPO_2 was 98% but tachypnea and tachycardia persisted despite normal body temperature and rehydration. At the 14th hour of hospitalization furosemid was administered due to anuria. On the 18th hour her blood pressure could not be measured, heart sounds were weak and peripheral circulation was impaired. Nasal O_2, dopamin and doputamine were started at once. Blood gasses showed compansated metabolic acidosis. Echocardiography showed pericardial effusion (2 cm width) and 30% ejection fraction. Pericardiosynthesis (40 ml) was performed but no improvement in the ventricular function was observed. Adrenalin infusion was started. She had cardiac arrest in the following three hours and she was unresponsive to cardiopulmnonary resuscitation.
Results: After her death serologic study confirmed measles infection (IgM positive, IgG negative). Blood culture remained sterile. Mother reported that the patient played with other children in the outdoors but she was not aware of any exposure with measles.
Conclusion: In Turkey vaccination rate for measles is about 90%. Due to conflict in Syria, vaccination was poor and more than 1.5 million immigrants came to Turkey. This resulted with ten-folds increase in measles. Myocarditis is among rare complications of measles. After transplantation generally fatal measles cases with encephalopathy or interstitial pneumonia are reported. HSCT guidelines recommend vaccination for HSCT recipients 24 months after HSCT. The majority of studies have shown that the response to measles vaccine occurs in approximately 70% of patients when administered >24 months post HSCT. Present patient was on the post-transplant 14th month. In Brazil during an epidemic, children were vaccinated 9-19 month post-transplant and it is reported that vaccination is safe and efficient. Treatment of measles is symptomatic. We are not sure if immunoglobulin administration would change prognosis. As a conclusion measles may be fatal in HSCT recipients in the second year of transplant. In the post-transplant 24 months, families must limit contacts of the HSCT receipents with other children.
References: 1. Ljungman P, Cordonnier C, Einsele H et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant 2009 44, 521–526
Disclosure of Interest: None declared.

P580
Abstract Withdrawn

P581
CMV genotypes in patiens after allogeneic haematopoietic stem cell transplantation

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Introduction: Human cytomegalovirus is one of the most important viral pathogens in patients after haematopoietic stem cell transplantation (HSCT). Better characterisation of CMV infections might give us the informations about impact of different CMV genotypes on the prognosis off the patient, including survival, presence of severe complications or risk of development of resistance and so help for better patient's tailored therapy in the future. Genotypisation was performed using UL55 gene (coding glycoprotein B) and UL74 gene (coding glycoprotein H).
Materials (or patients) and methods: We genotyped 1697 CMV positive samples from 135 children and 332 adult patients after allogeneic HSCT at Department of Paediatric Haematology and Oncology of Motol University Hospital and Institute of Haematology and Blood Transfusion between January 2002 and January 2013. DNA was extracted using Qiagen QiaAmp DNA Blood Mini and DNA Mini Kits from biological samples (from whole blood and in minor cases from other biological tissues). Samples were primary used for prospective CMV DNA testing and were subsequently stored at -20 °C. Genotypisation was performed using real-time PCR technology on Applied Biosystems 7500 and Bio-Rad CFX96 machines using specific primers and MGB-probes aimed at specific sequence of gB1-4 and gH1 and gH2 genotypes.
Results: CMV genotype was detected 1213 samples from 116 children and 297 adult patients. A single CMV strain was detected in 1,021 (84.17%) samples from 89 (76.72%) children and 200 (67.34%) adult patients. Mixed infection caused by two or more CMV strain was detected in another 192 samples (15.83%) from 27 children and 97 adult patients. Most frequently detected genotypes in ''single strain'' infection were gB1gH2 (detected in 350 samples from 25 children and

69 adults) and gB1gH1 (detected in 250 samples from 23 children and 44 adults). Most frequently detected strains in "mixed strains" infections were gB1gH1gH2 (in 41 samples from 21 patients) and gB1gB3gH1gH2 (in 43 samples from 19 patients). Likely due to long lasting storage and slow DNA degradation or due to enormously low CMV quantity in the samples, we were not able to detect any CMV genotype in samples from 54 patients.

Conclusion: Mixes CMV strain infections are quite frequent among patient s after allogeneic HSCT patients with higher frequency among adult patients. Detailed analysis of clinical consequences of viral infections including CMV are necessary for better understanding of both direct and indirect impact of the CMV on the outcome of HSCT recipients.

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Disclosure of Interest: None declared.

P582

Human Herpes Virus 6 Encephalitis: a major challenge after Haploidentical Hematopoietic Stem Cell Transplantation

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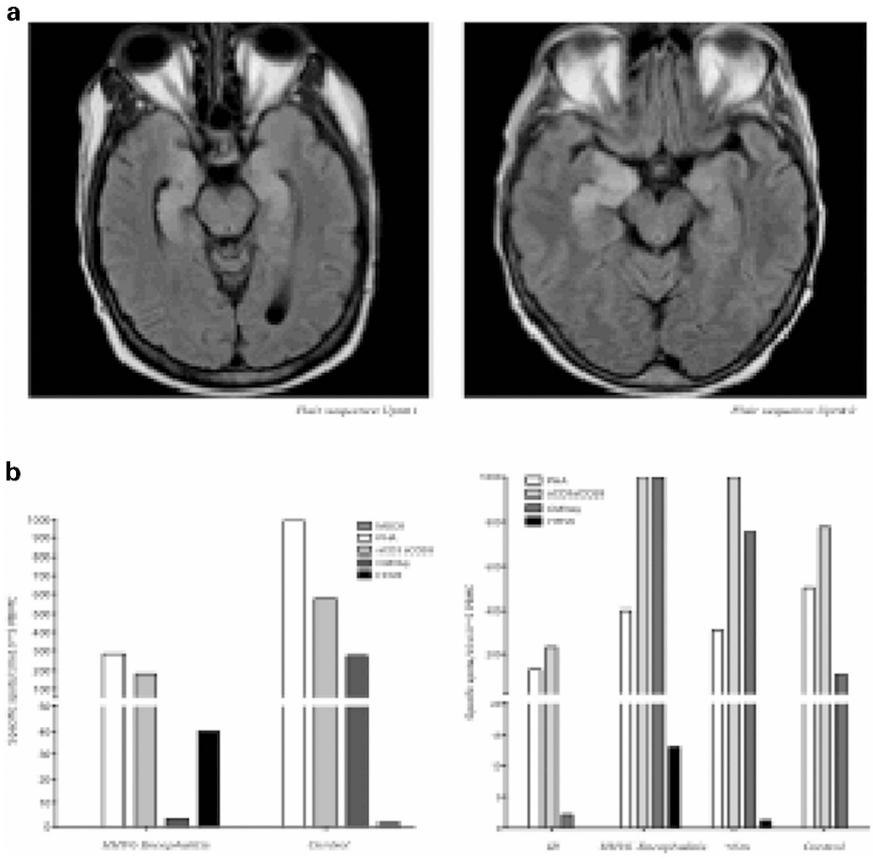
Introduction: Reactivation of human herpes-virus type 6 (HHV-6) represent a frequent occurrence after allogeneic HSCT from alternative donors, and has been associated with poor outcome. Still, little is known regarding the association between systemic HHV-6 reactivation and the development of encephalitis in the haploidentical setting.

Materials (or patients) and methods: From January 2009 to December 2014, our center recorded 12 adult patients (pts, median age 54 years) who developed HHV-6 encephalitis after allogeneic HSCT for high-risk hematological malignancies. Stem cell donors were all family haploidentical, using PBSC graft. Three pts received a T-cell depleted graft followed by the infusion of suicide gene-modified donor T-cells (TK cells). All the other pts received an unmanipulated PBSC graft, followed by GvHD prophylaxis with MMF and sirolimus. In vivo T-cell depletion with ATG-Fresenius was administered in 8 pts, while 4 pts received post-transplant cyclophosphamide. HHV6 viral load was determined by quantitative PCR (Nanogen Advanced Diagnostic S.r.L).

Results: Median time from haploidentical HSCT to HHV-6 encephalitis was 25 days (range: 2-218). Symptoms included: disorientation (12), confusion (12), memory loss (11), delirium, convulsion (5), hyponatremia (6), hypothermia (2), pruritus (4), consciousness loss (11), irritability (10), fatigue (12), headache (8). MRI revealed abnormal findings in 72% of pts, most commonly limbic encephalopathy with selective involvement of the medial temporal lobe (figure 1). High levels of plasma HHV-6 DNA were observed in all the pts, with a median viral load of 26719 copies/mL, with a direct correlation between peak plasma HHV-6 DNA and clinical symptoms. Cerebrospinal fluid examination was performed in half of the pts, demonstrating a median viral load of 64255 copies/mL. At the time of viral positivity all pts were receiving acyclovir as viral prophylaxis, except two. All patients received antiviral pharmacological treatment, using as first choice therapy foscarnet, even though the most four severe cases required a prolonged combination therapy with foscarnet and ganciclovir. Acute GvHD (grade I-IV) was concomitantly observed in

[P582]



7 pts, often requiring systemic steroids. A concurrent CMV positivity was detected in 4/12 pts. The median absolute count of CD3+ lymphocytes was 120 cells/mcl (0-1468). Amongst the six pts with documented immune-reconstitution (a median of 620 CD3+ cells/mcl for unmanipulated HSCT, and 207/mcl for pts treated with TK cells), five completely resolved the clinical event and are alive and disease-free at a median follow-up of 1.9 years from HSCT. All the remaining pts without a documented immune-reconstitution died, resulting in an overall mortality rate of 58%.
Conclusion: Although there is still a considerable uncertainty on the clinical significance of HHV-6 reactivation in literature, the mortality rate of HHV-6 encephalitis amounted to 58% in haploidentical HSCT, supporting the value of HHV-6 routine testing in this population. HHV-6 encephalitis should be considered in case of CNS symptoms. T-cell immune reconstitution is able to ensure a favorable outcome, clearly evident for patients treated with TK cells, also thanks to the efficient control of GvHD and the absence of post-transplant immune-suppression in this context.
Disclosure of Interest: R. Greco: None declared, M. Noviello: None declared, L. Vago: None declared, M. T. Lupo Stanghellini: None declared, N. Cieri: None declared, G. Oliveira: None declared, F. Giglio: None declared, M. Morelli: None declared, V. Valtolina: None declared, M. C. Barbanti: None declared, F. Lorentino: None declared, L. Crucitti: None declared, A. Orsini: None declared, A. Forcina: None declared, A. Assanelli: None declared, M. Carrabba: None declared, S. Marktel: None declared, M. Bernardi: None declared, C. Corti: None declared, J. Peccatori: None declared, C. Bordignon Employee of: Prof. Claudio Bordignon is an employee of MolMed S.p.A., Milano, Italy, C. Bonini Conflict with: Dr. Chiara Bonini is a scientific consultant of MolMed S.p.A., Milano, Italy., F. Ciceri: None declared.

P583
Microbiologically confirmed infections in the 2 years after haematopoietic stem cell transplantation

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Introduction: The immunocompromised state may persist for years after haematopoietic stem cell transplantation (HSCT) and infection is a major cause of morbidity and mortality in this group. We characterised all microbiologically confirmed infections, in terms of pathogen, phase, and outcome, in a cohort of patients followed up for two years after transplantation.
Materials (or patients) and methods: We reviewed all patients undergoing HSCT at a London teaching hospital over a 30 month period (April 2010 to September 2012). In addition to demographic information, data from the patients' medical records and the electronic microbiology results system were combined to capture all confirmed infectious pathogens within a 2-year observation period.
Results: There were 60 consecutive patients, 70% of whom were male, with a median age of 48 years (range 16-65). 25 patients received allogeneic transplants and 35 were autologous. There were 104 microbiologically confirmed infections, a mean of 1.7 infections per patient (range 0-6). Thirty-six infections (35% of the total) occurred in phase I (in the first 30 days post-transplantation or prior to neutrophil recovery), with 37 infections (35%) in phase II (engraftment to day 100) and 31 (30%) in phase III (day 100+). Phase I infections were evenly spread between bacterial and viral pathogens (18 and 17 respectively) with 1 parasite (*Toxoplasma gondii*). Phase II infections were mainly viral (29 episodes), with 6 instances of bacterial infection and 2 mycobacterial (*M. tuberculosis and abscessus*). Bacterial infections accounted for 19 of the 31 infections in phase III, with 10 cases of viral infections and 2 fungal (invasive Aspergillosis). A total of 33 different pathogens were identified, most commonly staphylococci, streptococci, enterococci, *Pseudomonas aeruginosa*, *E. coli*, *Clostridium difficile*, Epstein-Barr virus, cytomegalovirus, herpes simplex, and B.K. virus. There were 29 episodes of bacteraemia, ranging from Pseudomonas on day 3, to Listeria on day 411 post transplant.
Ten of the 60 patients (17%) died from infection within two years of HSCT. Three patients died in phase I from neutropenic sepsis caused by multidrug-resistant *Pseudomonas aeruginosa*. Another patient died prior to engraftment from reactivated toxoplasmosis which was diagnosed post mortem and had disseminated to the brain, heart and lungs. The one death in phase II of the post transplant period was from tuberculosis in a man originally from an endemic country. In phase III, five individuals died from hospital-acquired pneumonia, two of whom had concomitant invasive pulmonary Aspergillosis.
Conclusion: This cohort study demonstrates the importance of prolonged follow-up in defining the true burden of infectious complications after stem cell transplantation. Our patients were susceptible to a diverse range of pathogens across all phases of the post-transplant period and limiting the analysis to 100 days would have missed over a quarter of infections and infection-related deaths. Multidrug-resistant bacteria remain a threat following HSCT and empiric therapy for neutropenic sepsis should be tailored to the local microbiological environment. The toxoplasmosis death highlights the lack of protection against reactivation of this parasite with the use of nebulised pentamidine rather than oral co-trimoxazole for *Pneumocystis jirovecii* pneumonia prophylaxis during the pre-engraftment phase.
Disclosure of Interest: None declared.

P584
HHV-6 reactivation in 27 autologous hematopoietic stem cell transplant recipients

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Introduction: HHV-6 reactivation in allogeneic stem cell transplant recipients encompasses a broad variety of clinical manifestations ranging from asymptomatic reactivation to severe systemic infections and encephalitis. Reports on HHV-6 reactivation after autologous stem cell transplantation (AHSCT) are scarce. The objectives of this study were to describe signs and symptoms, associated factors, treatment and outcome of patients with HHV-6 reactivation.
Materials (or patients) and methods: This is a retrospective analysis of all adults, hospitalized in the lymphoma unit in St Louis hospital, with at least one whole blood positive quantitative polymerase chain reaction (PCR) DNA following AHSCT between January 2008 and January 2014. Demographics, haematological and AHSCT conditions, clinical and biological data were retrieved from the medical charts. Treatment and outcome were also analyzed.
Results: Twenty-seven cases were included. They were mainly men ($n=17$, 63%), median age 55 years (range 18-66). All patients received an AHSCT for a lymphoma (diffuse large B cell lymphoma in 12 cases, 44%). Conditioning regimen were BEAM (BCNU, etoposide aracytine, melphalan) in 70% of cases ($n=19$). In all patients HHV6 PCR was part of a diagnostic procedure implemented because of fever. Median time between AHSCT and first positive PCR was 13 days (range 9-25). Clinical manifestations were fever in all cases, respiratory signs in 9 cases (33%), diarrhea in 15 (56%), skin rash in 17 (63%). Thrombocytopenia requiring transfusion was noted in 16 cases (59%), neutropenia in 2 patients (7%), hepatic cytolysis (ALAT > 2 N) in 5 (19%). Chest scan ($n=11$) changes included alveolar ($n=4$, 36%) or interstitial syndrome ($n=3$, 27%). All Skin biopsies ($n=3$) showed histopathologic findings compatible with viral involvement. Median first whole blood HHV-6 DNA was 4.91 log per ml (range 2.92-6.56). Detection of HHV-6 DNA was positive in skin biopsies in 2 patients, in bowel biopsy in 2 patients and in bronchoalveolar lavage in 1 patient.

In 13 patients (48%) HHV-6 was considered as responsible for the fever and other signs, leading to a treatment in 9 cases (33%) (foscavir in 4 patients, ganciclovir in 4 and valganciclovir in 1). Fever and other signs and symptoms resolution was obtained in all patients. One patient required intensive care unit transfert. Median time to apyrexia from the beginning of treatment was 2 days (range 1-15). Median treatment duration was 25 days (range 11-60). Median HHV-6 DNA after treatment was 3,3 log per ml (range 0-4,81). Recovery was observed in all cases.

Conclusion: HHV-6 reactivation in AHSCT recipients was responsible for early fever, associated with diarrheas and skin rash in more than 50% patients and respiratory signs in 30%. It led to antiviral treatment in 30% of cases. Outcome was favorable in all patients. Prospective studies are warranted to identify risk factors of symptomatic HHV6 reactivation in this population and better target patients profile for treatment.

Disclosure of Interest: None declared.

P585

Evaluation of T-cell responses against transplant related infectious antigens in donor cells before allogeneic hematopoietic stem cell transplantation

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Introduction: Infectious complications in the immunosuppressed state after allogeneic HSCT can be associated with high morbidity and mortality, thus limiting the efficacy of the transplantation. The aim of the study was to assess whether already acquired immunity in the passenger lymphocytes from the donor graft can be protective in the recipient post transplant.

Materials (or patients) and methods: Cell composition and functionality in 4 bone marrow and 18 peripheral blood stem cell products obtained from 22 allogeneic donors were evaluated prior to transplantation. Lymphocyte lineage markers (CD3, CD4, CD8, CD19, CD56, CD16, TCR$\alpha\beta$ and TCR$\gamma\delta$), CD34-expression and T-cell proliferation against *Candida albicans*, CMV, EBV, HSV1, VSV and positive control Pokeweed mitogen (PWM) were analysed using flow cytometry and a modified version of the flow cytometry based proliferation assay FASCIA. FASCIA is a whole blood assay, where proliferating cells' blast formation is analysed. Proliferative responses were expressed as stimulation indexes. Specific T-cell responses to pathogens were compared to the recipient's clinical records.

Results: PWM stimulation elicited proliferation of T-cells from all donors. The frequency of donors in which we could detect T-cell responses to specific infectious antigens was the following: Candida; 77%, CMV; 36% EBV; 86%, HSV1; 18% and VZV; 45%, which correlated to donor serology for CMV, HSV and EBV. Ten month past transplant 11 of 22 recipients had experienced reactivation of either CMV HSV or VZV. There was no difference in T-cell proliferation against CMV, HSV or VZV in the donor grafts when comparing the two patient groups. Neither did leukocyte composition, i.e. frequency of granulocytes, monocytes, CD34+ stem cells, CD4+, CD8+ T-cells, NK-cells or B-cells differ between the two patient groups which reactivated CMV, HSV or VZV. However, the frequency of TCR$\gamma\delta$ T-cells was significantly higher in the group without infections, mean 3.85% (0.23- 9.53) compared to the group with infections, mean 1.86% (0.35-6.63).

Conclusion: We show that in allogeneic stem cell transplantation, donor stem cell products contain functional T-cells with different specificities for various infectious agents. These protective cells are probably transferred to the recipients, but due to immunosuppression and GVHD prophylaxis unable to function. In the patient group without reactivation of Herpes viruses post transplantation, the frequency of TCR$\gamma\delta$ T-cells was higher in the grafts compared to the group with infection, which may indicate a protective role of TCR$\gamma\delta$ T-cells. This study highlights the need to examine all cells in the graft, not only concentration of CD34+ cell, to possibly predict clinical outcome.

Disclosure of Interest: None declared.

P586

Multiple vaccination with a CMVpp65-derived peptide can prevent or clear CMVpp65 antigenemia after allogeneic stem cell transplantation

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Introduction: After allogeneic stem cell transplantation cytomegalovirus (CMV) can reactivate and cause serious complications like pneumonia, colitis and encephalitis. Moreover CMV has nephro- and myelotoxic effects. Patients testing seropositive for CMV are at high risk for reactivation. Therefore a vaccine against CMV is highly desirable, but not yet available. The CMV phosphoprotein 65 (CMVpp65) derived nonamer peptide NLVATVPMV has been well characterized as immunogenic. Therefore we designed a vaccine with 300 microgram of the peptide in an oil-in-water emulsion (Montanide™).

Materials (or patients) and methods: Ten CMV-seropositive patients after allogeneic stem cell transplantation for hematological malignancies received four applications of the vaccine s.c. at a biweekly interval. As adjuvans GM-CSF was applied subcutaneously on the two days preceding, the day of and the two days following peptide vaccination. Eight patients had already experienced several episodes of CMV viremia and obtained the vaccine as preemptive treatment manner, two patients received the CMVpp65 peptide vaccine as a prophylaxis. Patients were monitored for clinical course as well as CMVpp65 antigenemia. Before each vaccination and on at least two time points after the last vaccination peripheral blood of the patients was drawn and assessed by multi-color flow cytometry including tetramer staining for HLA-A2 and -B7 CD8+ T cells specifically recognizing the CMVpp65 antigen. Besides CD8+CCR7+CD45RA effector T cells also natural killer (NK) cells, regulatory CD4+ CD25hiFoxP3+ T cells as well as gamma/delta T cells were evaluated. ELISPOT assays were performed for the secretion of interferon gamma and granzyme B of CMV65-specific CD8+ T cells. Serological tests to evaluate dynamics in CMV-IgG and -IgM-titers were performed.

Results: Ten patients included in this clinical phase I study received 4 vaccinations each. As characteristic for Montanide™-based vaccines no side effects were observed with the exception of CTC grade I rash and induration of the skin at the site of injection. These side effects resolved. No other toxicities were observed. Seven of eight patients with CMVpp65 antigenemia cleared the CMV after four vaccinations and are free from viremia till present (maximum more than two years) despite cessation of antiviral prophylaxis. The two patients put on prophylactic vaccination never developed viremia. We could detect CMVpp65-specific CD8+ T cells in eight patients after vaccination. An increase of secretion of interferon gamma and granzyme B was observed. The frequency of regulatory T cells was decreasing in a part of the patients. The analysis of gamma-delta T cells is still pending and will be reported in the conference. As expected no serological responses were detected after the administration of a HLA-class I nonamer peptide.

Conclusion: Taken together, administration of CMVpp65 peptide in Montanide™ as vaccination was save and well tolerated in all patients. The clinical effects were definitively positive as the virus was cleared after vaccination. We were able to detect proliferating and activated CMV65 specific T cells. In summary CMVpp65 peptide vaccination constitutes an interesting option for patients at risk for CMV reactivation and

we recently extended this clinical trial also to hemodialysis patients before kidney transplantation.
Disclosure of Interest: None declared.

P587

Multiple virus infections in pediatric allogeneic transplant recipients including Adeno-, Cytomegalo- and Epstein-Barr virus reactivations

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Introduction: To identify viral risk factors that might influence the outcome of pediatric allograft recipients (allo HSCT), we analysed not only Adenovirus (AdV) infections but rather the relevance of multiple virus reactivations of additionally Cytomegalovirus (CMV) and Epstein-Barr virus (EBV).
Materials (or patients) and methods: 167 subsequent pediatric allogeneic stem cell transplant recipients were analyzed. Transplant indications included malignant (myeloid, $n=$ 55; lymphatic, $n=$ 51) as well as nonmalignant diseases ($n=$ 61). Viral DNA was monitored on a weekly basis from stool (AdV) by PCR and plasma (AdV, CMV and EBV) by quantitative PCR.
Results: 52 of 167 children (31%) were tested positive for AdV DNA in stool and 31 patients (60%) of these 52 had AdV viremia. Of these 31 children, 21 deceased. In 52 children tested positive for AdV DNA in stool samples an AdV C species could be determined in 26 cases and an AdV A in 3 cases. In the 31 cases with viremia, an AdV C species was detected in 16 patients. Four children (25%) carried different C species in stool and in plasma. AdV as the only virus was detectable in eleven of 52 patients in stool and/or in plasma with an overall mortality of 36% (4 cases). In 28 children, two viruses (AdV and either CMV or EBV) were detected with a TRM rate of 50% (14 cases). The TRM rate of 69% was the highest among 13 children that carried all three viruses (nine deaths). 24 of 52 children with AdV viremia (46%) revealed that donors and recipients were both CMV IgG seropositive, whereas this was the case in 31 of the remaining 115 AdV negative patients (27%).
Conclusion: The AdV reactivation rate of about 30% in allogeneic transplant recipients in our pediatric cohort was in accordance with findings by other groups. AdV C species predominated, followed by AdV A12. No AdV B or any other AdV species were detected. AdV viremia was clearly associated with a higher mortality rate. The underlying disease was of no relevance. Also the reactivation rates of CMV and EBV as a single virus were in the well known and expected range. The most striking finding was the highly serious effect of multiple virus reactivations.
Especially regarding the simultaneous reactivation of multiple viruses a better pre-emptive therapy and/or prophylactic approach is required including antiviral drug combinations and adoptive treatment strategies. But also a better understanding of virus - virus as well as virus - immune system interactions seems to be mandatory.
Disclosure of Interest: None declared.

P588

Upper and lower respiratory tract infections by respiratory viruses in adult recipients of allogeneic hematopoeitic stem cell transplantation (Allo-HSCT): Single center

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Introduction: Respiratory viruses are known to be the major causes of morbidity and mortality in recipients of Allo-HSCT. In this study, we evaluated the results of respiratory viral panel in the patients after Allo-HSCT, having the symptoms and/or findings of upper or lower respiratory infections.
Materials (or patients) and methods: In our hospital, we initiated to perform respiratory viral panel in the patients having the symptoms and/or findings of upper or lower respiratory infection since January 2013.Therefore, we included totally 25 patients (15 M/10 F) who underwent allo-HSCT for benign or malign hematological disease.Median age was 40 years (range, 20-63).Nasopharyngeal aspirates were used for obtaining upper respiratory specimens for respiratory viral panel.Viral panel was studied withmultiplex PCR method with "Fast –Tract Diagnostics Respiratory Pathogens 21"(Luxemburg). Adenovirus,Corona virus 229E/NL63,OC43/HKU1,Parainfluenza virus 1,2,3,4,Rhinovirus,Influenza A,B and H1N1 virus,Respiratory Syncytial virus (RSV),Boca virus,Metapneumovirus A/B, Enterovirus,Parechovirus and Mycoplasmapneumoniae can be detected by this technique.
Results: We detected the viral panel positivity in 18 patients with median 31.5 days (range: 8-607 days) after the transplantation. The most frequent viral agent disclosedwas RSV (33.3%; $n=6$) followed by Rhinovirus ($n=5$), Coronavirus ($n=4$), Influenza ($n=3$),Parainfluenza ($n=3$), H1N1 ($n=1$), Metapneumovirus ($n=1$) and Adenovirus ($n=1$). In six patients two viruses were detected concurrently (2 Rhinovirus plus influenza; 2 RSV plus Coronovirus; 1 Rhino plus RSV; and 1 Coronovirus plus influenza). Although CMV reactivation was occurred in 3 patients at the time of viral panel positivity, there was no statistical correlation between CMV reactivation and respiratory viral panel positivity ($P=0.25$). Most of the patients ($n=17$) with viral panel positivity were under immunosuppressive therapy for graft versus host disease prophylaxis or treatment.In 4 of 7 viral panel negative patients, bacterial infections were accompanied.In addition, when compared the results of theviral panel with the lymphocyte count and the absolute neutrophil count, serum CRP and serum albumin level, we did not find any statistical differences (Table 1).

Table 1. The comparisons of the other laboratory parameters in the patients with viral panel positive with the negative ones.

Patients	*Lymphocyte count (10e6/L), (range)*	*Neutrophil count (10e6/L), (range)*	*CRP mg/dl, range*	*Albumin g/dl, range*
Viral panel positive	350(0-6500)	1500 (0-6300)	103.7 (1-300)	2.75 (2-3.8)
Viral panel negative	600 (0-2300)	1700 (0-10800)	24 (2-174)	3.2 (2-3.7)
p	0.7	0.4	0.5	0.4

Conclusion: Although viral panel positive patients had lower lymphocyte and neutrophil count with higher CRP, the difference was not found to be statistically significant. CMV reactivation is not related with viral panel positivity. In viral panel negative patients, high CRP levels might show infections rather than virus in etiology. The limitations of our study were that few patients were able to be evaluated in, and also the evaluations had both early and late time-period after the transplantation. Nevertheless, we thought that the results should give an opinion about the development of respiratory viral infectionsto the clinicians.
Disclosure of Interest: None declared.

P589

Comparison of teicoplanin serum concentrations of three teicoplanin regimens in adult haematology patients

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Introduction: The glycopeptide antibiotic teicoplanin is used in the treatment of serious Gram-positive infections, including methicillin-resistant *Staphylococcus aureus* (*S. aureus*) infections.

It is also used in the management of neutropenic sepsis in patients with haematological malignancy, in particular in patients with indwelling intravascular access. Recent data showed a positive correlation between treatment outcome with teicoplanin for *S. aureus* septicaemia and serum trough concentrations. Furthermore, rapid achievement of target trough concentrations reduced the risk of the development of microbiological resistance to teicoplanin and treatment failure.
Survey data suggest that therapeutic drug monitoring of teicoplanin is not routinely carried out in the UK and Ireland. The aim of this study was to determine teicoplanin serum levels of 3 different dosing regimens in haematology patients.
Materials (or patients) and methods: Teicoplanin serum levels were monitored in patients with febrile neutropenia following chemotherapy or haematopoietic stem cell transplant. Patients received teicoplanin at different dosing regimen in combination with piperacillin/tazobactam and amikacin. Serum levels were analysed by a polarization fluoro-immunoassay. Target trough concentrations (C (min)) were defined as >10 mg/l at 24 hours, 15-20 mg/l at 48 hours and >20 mg/l at 120 hours.
Group 1: 4 patients (NHL 1, Burkitt's 1, AML 2). Teicoplanin was administered at the following dosing regimen which was the standard regimen at our institution:
<85 kg: 400 mg intravenous (IV) 12-hourly for 3 doses, then 400 mg IV once daily >85 kg: 600 mg IV 12-hourly for 3 doses, then 600 mg IV once daily
Serum levels were taken at 24, 48, 96 and 120 hours after the first dose administered.
Group 2: 6 patients (AML 2, MDS 1, NHL 2, Hodgkins 1). Patients received teicoplanin according to the Outpatient Antibiotic Therapy regimen used at our institution: 800 mg IV once, followed by 600 mg IV once daily (<80 kg) or 800 mg IV once daily (>80 kg). Serum levels were taken at the same time points as in Group 1.
Group 3: 5 patients (AML 2, MM 2, NHL 1). Teicoplanin was administered as follows: 10 mg/kg IV 12-hourly for 3 doses followed by 10 mg/kg IV once daily. Serum levels were taken at 48 and 120 hours.
Results: Group 1: C (min) >10 mg/l at 24 hours and C (min) 15-20 mg/l at 48 hours was only achieved in 1 patient. 2 patients had C(min) >20 mg/l at 120 hours; however, 2 patients still had C(min) <10 mg/l at 120 hours.
Group 2: 2 patients had C (min) >10 mg/l at 24 hours, C (min) 15-20 mg/l at 48 hours and C(min) >20 mg/l at 120 hours. 3 patients had C(min) <10 mg/l at 48 hours. 1 patient still had C (min) <15 mg/l at 120 hours.
Group 3: 1 patient had C (min) 15-20 mg/l at 48 hours. 2 patients had C(min) >20 mg/l at 120 hours. 3 patients had C(min) 10-15 mg/l at 48 hours and C (min) 15-20 mg/l at 120 hours
Conclusion: Our study showed that the higher loading dose regimen (Group 3) achieved more effective therapeutic serum concentrations of teicoplanin. However, serum concentrations varied strongly between individual patients most likely due to altered pharmacokinetics of teicoplanin observed in patients with haematological malignancy. Therefore, routine steady state therapeutic drug monitoring is recommended to optimize individual teicoplanin therapy.
Disclosure of Interest: None declared.

P590
The rapid nursing initiation of the first line antibiotics for neutropenic patients experiencing pyrexia and/or suspected sepsis: using patient group direction (PGD) improves patient outcome and experience

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Introduction: For every hours delay in septic patients receiving intravenous antibiotics the chances of survival reduce by 7.6% (Kumar et al 2006). This is likely to be even greater in the neutropenic population. A review of the time it took haematology / HSCT patients to receive first line antibiotics at UCLH in London ('Spike to needle time') for suspected neutropenic sepsis identified significant delays over and above the recommended 60 minute maximum. Delays were mainly as a result of the wait for busy doctors to attend the patient. While these delays often occurred at night, surprisingly, delays also occurred in daytime hours. To reduce this critical period, the service developed and introduced a Patient Group Direction (PGD) in order for haematology nurses to initiate and administer the first line antibiotic of Tazobactum or Ceftazadine.
Materials (or patients) and methods: A pre and post intervention clinical audit of first line antibiotic delivery was conducted across haematology in-patient units. Haematology / HSCT staff were surveyed regarding the use of PGD and their level of competency. A competency based training resource was developed and the antibiotic-PGD was mandated across the service.
Results: Pre-PGD, first line antibiotics were appropriately delivered 46% of the time.
The nursing survey reported 80% non-PGD givers would like to be trained, 85% would like to see more PGD prescribers and 70% wanted more PGD education. One year on the average time to 1st line antibiotic delivery has dropped to 26 minutes, based on a revised target time of 30 minutes ($n=200$). 'Spike to Needle' times reduced significantly and have been maintained with 100% nursing administration.
Conclusion: These improvements only served to highlight continued delays in the delivery of 2nd line antibiotic (Gentimycin or Ciprofloxacin). A second PGD has subsequently been developed for 2nd line antibiotics. To address concerns of nurse prescribing of nephrotoxic antibiotics the latest PGD includes an electronic algorithm that nurses can use to safely determine dosage.
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Disclosure of Interest: None declared.

P591
Retrospective study about infections in patients with multiple myeloma during first month and one year after autologous stem cell transplantation

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Introduction: Autologous transplantation of hematopoietic stem cells (ASCT) remains the standard for treatment of patients with multiple myeloma (MM), leading to an increased overall survival. Despite the advances in antimicrobial prophylaxis, infections are still present. The aim of this study was to analyze the number of febrile episodes, the incidence and type of infections in the early period and during the first year after ASCT and also their impact on survival.
Materials (or patients) and methods: Retrospective study of 56 patients diagnosed with MM submitted to a first ASCT between January 2010 and December 2013 in our institution. All patients received antimicrobial prophylaxis with levofloxacine, acyclovir, fluconazole and pentamidine. We collected clinical data, the number of febrile episodes, microbiologically documented infections (MDI), the origin of fever and the rate of bacteremia in the first month and during the first year post-ASCT.
Results: The median age of the series was 59 years (29-69). 39 patients were male (70%). All patients had been treated with bortezomib and 79% were in partial remission at the time of ASCT. Conditioning regimen was melphalan 200 mg/kg (or 140 mg / kg in renal failure) and in all G-CSF was administered from day $+7$ for a median of 6 days (3-9).). Median days to neutrophil recovery ($>$ 500 / uL) was 12 (7-17). 48 patients (85%) had a febrile episode during the early period, reaching the median on day $+7$ (2-14), and one of three had MDI; only one patient died from sepsis by *E. faecium*. Infections in the early and

the late period post-ASCT are described in table 1. Thoughout the year, two patients had a viral infection by *herpes simplex,* and no patient had documented fungal infection.

Table 1. Description of febrile episodes and bacteriological infections in both periods (CNS: coagulase negative *staphylococcus*; HSC: hepatosplenic candidiasis)

Post ASCT-period	*EARLY*	*LATE*
Febrile episodes	46/56 (82%)	12/56 (21%)
DMI	16/48 (33%)	3/12 (25%)
Bacteriemia	3/48 (6%) - E.faecium 1/16 - E. coli 1/16 - S. epidermidis 1/16	2/12 (16%) - S. pneumoniae 1/2 - C. koserii 1/2
Bacteriological Infections	16/48 (33%) - S. epidermidis 6/16 (37%) - *S. aureus* 1/16 (6%) - CNS 2/16 (12%) - *E. coli* 3/16 (19%) - *P. aeruginosa* 2/16 (12%) - *Candida sp* 1/16 (6%) - E.faecium. 1/16 (6%)	3/12 (25%) - S. pneumoniae 1/3 (33%) - *C. koserii* 1/3 (33%) - *E. coli* 1/3 (33%)
Fever origin	- Sepsis 3/16 (20%) - Catheter 5/16 (32%) - Urinary 2/16 (12%) - Enterocholitis 2/16 (12%) - Respiratory 2/16 (12%) - Pneumonia 1/16 (6%) - Possible HSC 1/16 (6%)	- Sepsis 3/12 (25%) - Respiratory 3/12 (25%) - Pneumonia 4/12 (33%) - Urinary 2/12 (17%)

Conclusion: Myeloma multiple patients receptors of a ASCT had a high incidence of fever in the early period, with MDI in one third of cases, and a low frequency of sepsis. In our experience, the frequency of infections and mortality due to infection during the first year post ASCT was very low.

Disclosure of Interest: None declared.

P592

Low-dose cidofovir treatment of BK virus-associated hemorrhagic cystitis in recipients of hematopoietic stem cell transplant in acute leukemia patient

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Introduction: The association of BK virus infection with hemorhagic cystitis (HC) in blood and marrow transplant (BMT) recipients was first demonstrated two decades ago. HC complicates the course of 7-40% of recipients of Hematopoietic Stem Cell Ttansplants (HSCT); early onset disease is probably secondary to the conditioning regimen and later onset disease has been associated with viral pathogens. HC is a significant cause of morbidity after HSCT with different grades of severity related to the degree of hematuria.

Materials (or patients) and methods: Between June 2011 and November 2014, 39 allogenic HSCT were performed at the University of Uludag, Turkey. A total of 5 samples obtained from 39 post-transplanted recipents for the development of hematuria (GII-GIV) were included to the study. Retrospective screening consisted of clinical and laboratory assesment and BKV urine and plasma PCR (Table I).

Results: In our institution, HSCT recipients with BKV-associated HC are treated with 1 mg/kg of cidofovir weekly. We identified HSCT recipients with BKV-associated HC, treated with weekly cidofovir. Five allogeneic HSCT patients received a mean of 5-6 weekly doses of cidofovir. HC occurred at a mean of 69 days after transplant. A clinical response was detected in 5/5 patients, and microbiologic response was defined as at least one log reduction in BKV viral load by qPCR and was assayed within 2 weeks of completion of treatment.

Conclusion: Currently, there is no standart treatment for BK virus-associated HC, but there are reports that cidofovir is effective in a dosage of 5 mg/kg once weekly. We conclude that weekly low dose cidofovir appears to be a safe treatment option for BKV-associated HC.

Disclosure of Interest: None declared.

P593

Risks and outcomes of invasive fungal infections in the first six months after allogeneic hematopoietic stem cell transplantation in pediatric patients: a Multicenter Cohort Study by the Turkish Pediatric Bone Marrow Transplantation Study Group (TPBMT-SG)

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[P592]

Table 1. Characteristics of patients treated with cidofovir

Age/sex	*Diagnosis*	*Time of HSCT/ Conditioning regimen*	*Time of engraftment (day)ANC less than 500x10[6]*	*Time of HSCT (day)*	*İmmunosupressive treatm.Cyc/ Steroid/ Mycophenolate*	*Peak plasma BKV load (x10[6])/Grade HC*	*Start of treatment/ Time*	*BKV load of treatment/ Microscopic hematuria*
43/M	AML-M2 2. CR	23.06.11 Bu/Cy	16	+43	Cyc/Steroid	Positive / GIV	10.11.11/ 6 week	Negative/1
21/F	AML-M2 2.CR	13.10.11 Bu/Cy	27	+225	Cyc/Steroid	48900/GIII	12.06.12/6 week	Negative/1
51/F	MDS-AML-M4 1.CR	20.02.13 Bu/Cy	12	+33	Cyc	>5000x10[6]/ GII	04.04.13/5 week	<5000/1
39/M	AML-M2 1. CR	15.03.13 Bu/Cy	22	+69	Cyc	863000/ GII	10.06.13/6 week	Negative/1
26y/M	Ewing-AML 2. CR	09.03.13	Another center	+81	Cyc/Steroid/ Mycophenolate	50x10[6]/GIV	31.05.2012/ 6 week	Negative/1

*2 weeks after the completion of the treatment were evaluated

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Introduction: Invasive fungal infections (IFIs) are a major cause of infectious morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Because of limited data, the extend of this problem in children is not clear.
Materials (or patients) and methods: We retrospectively analyzed the incidence, outcome and risk factors of IFI in the first six months after 419 allogeneic HSCT in 412 pediatric patients treated at 16 different centers in Turkey from 1st of January 2013 to 31st of December 2013.
Results: The incidence of IFI in the first six months after allogeneic HSCT in pediatric patients was 10%, consisting of possible, probable and proven IFI at rates of 4%, 2% and 4%, respectively. IFI developed in 41 patients, 33 of which within 100 days, 19 of which occurred in the first month after transplantation. Among all 419 patients, 81 (19%) died after a median 60 days following transplantation (range, 8-180 days). Among 81 patients who died, 8 died due to or associated with IFI. Overall mortality rate attributable to IFI was 2% (8/419) and IFI was responsible or co-responsible for 10% (8/81) of deaths. Although invasive mold infections ($n = 6$ proven mold infection) appear to be more difficult to resolve (33%, 2/6) than invasive yeast infections ($n = 10$ proven yeast infection) (80%, 8/10), the power is lacking for statistical significance. Non-relapse mortality was not significantly different for patients with IFI and those without IFI. Univariate analysis showed that second transplantation ($P = 0.023$), unrelated donor ($P = 0.042$), late or none lymphocyte engraftment ($P = 0.003$), grade II-IV acute graft versus host disease (aGVHD) ($P = 0.002$), corticosteroid dose > 2 mg/kg/day for at least 10 days ($P = 0.021$), CMV reactivation ($P = 0.001$), ATG ($P = 0.001$) and Fludarabine ($P = 0.028$) were possible risk factors for IFI. Of these factors, grade II-IV aGVHD [relative risk (RR) of IFI = 2.801, $P = 0.008$], ATG (RR of IFI = 3.077, $P = 0.009$), late or none lymphocyte engraftment (RR of IFI = 2.451, $P = 0.013$), second transplantation (RR of IFI = 5.555, $P = 0.037$) and CMV reactivation (RR of IFI = 2.110, $P = 0.046$) were the factors associated with IFI in multivariate analysis.
Conclusion: The incidence and treatment success of IFI in this cohort are comparable to the rate reported in adult and other pediatric patients. Our results suggest that IFI after allogeneic HSCT in children is associated with grade II-IV aGVHD, second transplantation, CMV reactivation and late or none lymphocyte engraftment. Effective anti-fungal prophylaxis for these patients should be considered.
Disclosure of Interest: None declared.

P594

Therapeutic and Supratherapeutic Doses of Letermovir Do Not Prolong the QTc Interval in Healthy Subjects

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Introduction: Letermovir (MK-8228) is a potent, once-daily inhibitor of the cytomegalovirus (CMV) terminase complex that is being developed for the prophylaxis of CMV infection in transplant patients. This study evaluated the effect of therapeutic and supratherapeutic letermovir concentrations on cardiac repolarization in healthy female subjects
Materials (or patients) and methods: The trial was a randomized placebo controlled, 4-period, crossover trial designed to evaluate the effect of administration of a single intravenous dose of letermovir (960 mg supra-therapeutic dose and 480 mg clinical dose) on the corrected QT interval (QTc) in 38 healthy female subjects. The trial was double-blinded with respect to MK-8228 and placebo. Moxifloxacin was administered as a single 400 mg dose orally in an open-label fashion as a positive control. Each dose was followed by cardiodynamic measurements and pharmacokinetic sampling. Safety was monitored throughout the study by repeated clinical and laboratory evaluations.
Results: Administration of a single intravenous dose of 960 mg of letermovir does not prolong the QTc interval to a clinically significant degree. Specifically, the true mean difference (letermovir-placebo) of QTc change from baseline is less than 10 msec and the upper limit of the 90% CI for the maximum mean baseline-corrected difference from placebo is 7.53 msec (at 1 hour postdose). Similarly, administration of a single planned intravenous dose of 480 mg of letermovir (the clinical dose of letermovir) did not prolong the QTc interval to a clinically significant degree. The C_{max} for the 960 mg IV dose of letermovir was 67,900 ng/mL, providing a 2-fold margin to the C_{max} of the 480 mg clinical dose, which was 33,000 ng/mL when given intravenously in this study. Administration of moxifloxacin (positive control) was associated with an increase in QTc interval greater than 5 msec for all relevant time points with the lower bound of the 90% CIs ranging from 5.25–10.4 msec from 1-4 hours postdose, establishing assay sensitivity for this QTc trial.
Conclusion: Single dose administration of letermovir at the clinical dose of 480 mg and the supratherapeutic dose of 960 mg was safe and well tolerated and did not prolong the QTc interval in healthy female subjects.
Disclosure of Interest: W. Marshall Employee of: Merck and Co. Inc, F. Liu Employee of: Merck and Co. Inc, A. van Schanke Funding from: Merck and Co. Inc, G. Baheti Funding from: Merck and Co. Inc, W. Heber Funding from: Merck and Co. Inc, R. Goldwater Funding from: Merck and Co. Inc, Employee of: PAREXEL, B. Kantesaria Employee of: Merck and Co. Inc, C. Swaanen Employee of: Merck and Co. Inc, C. R. Cho Employee of: Merck and Co. Inc, J. Luk Employee of: Merck and Co. Inc, E. Hulskotte Funding from: Merck and Co. Inc, J. R. Butterton Employee of: Merck and Co. Inc, E. E. Marcantonio Employee of: Merck and Co. Inc.

P595

Multiple Intravenous and Multiple High Oral Doses of Letermovir (MK-8228) are Safe and Well Tolerated in Healthy Subjects

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Introduction: Letermovir (MK-8228) is a potent, once-daily inhibitor of the cytomegalovirus (CMV) terminase complex that is being developed for the prophylaxis of CMV infection in transplant patients. This study evaluated the safety and local tolerability of multiple daily intravenous doses of the cyclodextrin formulation of letermovir and the effect of multiple high oral doses of letermovir.
Materials (or patients) and methods: This was a 2 part, randomized, double-blind, placebo-controlled study to assess safety, tolerability and pharmacokinetics of letermovir in healthy female subjects. In Part 1, an oral dose of 720 mg of letermovir twice daily for 14 days was given. In Part 2, 480 mg

intravenous doses of the letermovir cyclodextrin formulation were given once daily for 7 days. Each part contained pharmacokinetic sampling. Safety was monitored throughout the study by repeated clinical and laboratory evaluations.
Results: Part 1: 17 subjects received oral doses of 720 mg letermovir twice daily for 14 days (single dose on Day 15) and 7 subjects received placebo. Letermovir exposures with this regimen were 4.6-fold higher than the exposures at the clinical dose of 480 mg daily. Oral administration of 720 mg letermovir twice daily was reasonably well tolerated. The most common adverse events (AEs) reported belonged to the gastrointestinal disorders system organ class that included vomiting, diarrhea, and abdominal pain or discomfort (89% of letermovir subjects and 33% of placebo subjects), followed by nervous system disorders that included dizziness, headache and presyncope (in 56% of letermovir subjects and 33% of placebo subjects). One subject had an elevation in transaminases and alkaline phosphatase at the end of 15 days of therapy that was concurrent with acetaminophen use and a viral illness; these elevations resolved within 2 weeks of the end of letermovir dosing. There were no deaths, severe AEs or discontinuations due to AEs.
Part 2: 9 subjects received 480 mg letermovir IV (cyclodextrin formulation diluted in 0.9% saline) daily for 7 days and 3 subjects received placebo. Letermovir exposures with this regimen were comparable to the exposures for oral letermovir administered at the clinical dose of 480 mg daily. Six of 9 (66.7%) subjects reported IV infusion related AEs after letermovir, and 2 of 3 (66.7%) subjects reported IV infusion related AEs after placebo. Infusion-related AEs included catheter site phlebitis (mild) and catheter site edema (mild) and one incidence of catheter pain that was moderate in severity. No cases of thrombophlebitis were reported. Two of 9 (22.2%) of the subjects reported GI related AEs while receiving IV MK-8228, including vomiting, diarrhea (soft stools), abdominal pain, abdominal discomfort and constipation; no GI related AEs were reported in the subjects who received IV placebo. IV administration of 480 mg letermovir daily was well tolerated. There were no deaths, SAEs, severe AEs or discontinuations due to AEs.
Conclusion: Letermovir is safe and generally well tolerated when administered either as oral doses of 720 mg of letermovir twice daily for 14 days or when administered intravenously in the cyclodextrin formulation for 7 days at the clinical dose of 480 mg of letermovir daily.
Disclosure of Interest: W. Marshall Employee of: Merck and Co. Inc, F. Liu Employee of: Merck and Co. Inc, A. van Schanke Funding from: Merck and Co. Inc, J. Udo de Haes Employee of: Merck and Co. Inc, G. Baheti Funding from: Merck and Co. Inc, W. Heber Funding from: Merck and Co. Inc, R. Goldwater: None declared, B. Kantesaria Employee of: Merck and Co. Inc, C. B. Smith Employee of: Merck and Co. Inc, C. R. Cho Employee of: Merck and Co. Inc, E. Hulskotte: None declared, J. R. Butterton Employee of: Merck and Co. Inc, E. E. Marcantonio Employee of: Merck and Co. Inc

P596
Inflammation and sepsis as part of hematopoietic stem cell transplantation: a transcriptomic approach

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Introduction: Patients with malignant hematological diseases and candidates for autologous stem cells (ASCT), are subject to neutropenic period sometimes complicated by sepsis. The severity of the infection depends on several factors, including the patient's pre-inflammatory condition, genetic factors, as well as the depth and duration of neutropenia. In the literature, inflammatory and infectious factors are often confused, as a predictive transcriptomic signature of sepsis has not been clearly demonstrated. Through a transcriptomic analysis of peripheral blood mononucleated cells, followed a bioinformatics and statistical analysis, we hope to identify predictive markers of sepsis development and/or severity.
Materials (or patients) and methods: A kinetic transcriptome analysis of peripheral blood mononuclear cells of 40 patients benefiting from autologous transplantation was performed. This research was approved by the ethical committee of our institution "Comité de Protection des Personnes Aix -Marseille II". Patients were included after informed consent was obtained. Peripheral blood was sampled before conditioning chemotherapy, then just before graft infusion and then after the end of aplasia. RNA was extracted, its quality checked and then samples were processed for use on Agilent microarrays following manufacturer instructions.
Results: Our study highlighted different expression profiles according to the chemotherapeutic regimen and the development of sepsis or not. Through a statistical analysis and with a false discovery rate (FDR) of 5%, 615 genes potentially predictive (48 h before the development of sepsis) are differentially expressed between patients who developed sepsis and those who have not developed.
Conclusion: The study of transcriptomics from a large number of patients allowed us to test our hypothesis and to confirm the potential predictive value of some sets of genes. Microarray results will be strengthened through quantitative PCR and validated by the dosage of any molecules of interest, which would allow the transfer of our experimental data in clinical practice.
Disclosure of Interest: None declared.

P597
Incidence and risk factors associated with hepatitis B virus clearance in HBsAg positive recipients undergoing allogeneic stem cell transplantation

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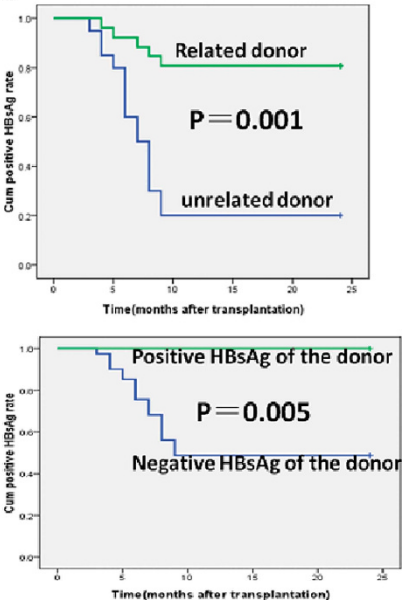
Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) results in a transfer of ''naive'' and specific memory cells. Previous report demonstrated the clearance of chronic hepatitis B virus (HBV) infection (seroconversion to nondetectable HBsAg) by way of allo-HSCT. Nonetheless, some HBsAg + recipients still kept positive for HBsAg post-transplantation. Here we present the first retrospective study to determine the incidence and risk factors associated with HBV clearance in HBsAg + recipients undergoing allo-HSCT in order to boost HBV clearance rate.
Materials (or patients) and methods: Between 2005 and December 2013, 46 HBsAg + patients undergoing allo-HSCT were enrolled. HBV clearance was defined as positive HBsAg disappeared within 12 months post allo-HSCT. 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. Donor type, HLA disparity, incidence of aGVHD and cGVHD, HBsAb level in donor, primary disease, conditioning regimen containing ATG and sex disparity were analyzed.
Results: Median follow-up time was 44 months (12-96). 21 of 46 (45.7%) patients converted to negative HBsAg and the other 25 patients (54.3%) still kept positive for HBsAg. In HBsAg + recipients with HBsAg + donors, all 5 recipients (100%) kept positive for HBsAg after allo-HSCT. In converted HBsAg negative recipients 5 received hematopoietic

[P597]

a

Characteristics	HBV clearance P value
Univariate analysis	
Age	0.944
Gender	0.854
Diagnosis	0.543
Donor type	0.001
HLA-mismatch	0.716
Incidence of aGVHD	0.897
Incidence of cGVHD	0.789
HBsAb level in donor	0.656
Conditioning regimen containing ATG	0.767
Donor HBsAg status	0.001
Univariate analysis	
Donor type	0.001
Donor HBsAg status	0.005

b



stem cells from related donors (23.8%) compared with 16 (76.2%) from unrelated donors which were statistically different ($P = 0.001$)(Fig1A,B). At 60 days cumulative incidence (CI) of neutrophil recovery was 86.8 ± 5% (HBV clearance group VS non-HBV clearance group CI: 84.9% VS 86.6 $P = 0.893$). CI at 100 days of grade II-IV aGvHD was 36 ± 4.7% (HBV clearance group VS non-HBV clearance group CI: 36.7% VS 38.6% $P = 0.765$). Based on the results of univariate and multivariate analyses, donor type ($P = 0.001$) and donor HBsAg status ($P = 0.005$) were significantly correlated with HBV clearance while primary disease type, donor and recipient's sex, HBsAb level in donors and conditioning regimen containing ATG were not associated with the seroconversion statistically(Fig1a,b).

Conclusion: Our data firstly suggests that unrelated donor type and HBsAg negative donor are the 2 clinical factors that makes the HBsAg positive recipients more proned to HBV clearance post allo-HSCT. For HBsAg positive transplant, unrelated and HBsAg negative donor will be preferred.

Disclosure of Interest: None declared.

Early complications / late effects & quality of life II

P598

Salvage therapy for primary graft failure after hematopoietic stem cell transplantation; a single center cohort study

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Introduction: Primary graft failure (PGF) is a significant complication following hematopoietic stem cell transplantation (HSCT). It greatly affects the prognosis whatever we perform donor lymphocyte infusion, boost infusion of donor hematopoietic cells, or salvage HSCT. In our institution, bone marrow aspiration is routinely performed every week until day + 28 after HSCT to perceive the early signs of PGF, and PGF is defined as the absence of engraftment signs on day + 21 and + 28. We aimed to evaluate the clinical impact of PGF in HSCT.

Materials (or patients) and methods: We reviewed medical records of 314 consecutive allogeneic HSCT for 291 patients with malignant disease performed in our institution from January 1990 to May 2014. During study period, 8 patients in 9 HSCT developed PGF. The underlying diseases of these 8 patients consisted of acute lymphoblastic leukemia (ALL, $n = 4$), acute myelogenous leukemia (AML, $n = 2$), juvenile myelomonocytic leukemia (JMML, $n = 1$), and malignant lymphoma (ML, $n = 1$). Of 8 patients with PGF, 4 patients received bone marrow transplantation (BMT) from sibling ($n = 1$), parents ($n = 2$), and an unrelated volunteer ($n = 1$); the other 4 patients with unrelated cord blood transplantation (u-CBT). Five patients were treated with myeloablative regimen, and the other 3 were prepared with non-myeloablative one. We analyzed the prognosis factors for outcomes after PGF.

Results: One patient with AML died at day + 55 after HSCT because of multi-organ failure induced by hepatic veno-occlusive disease without neither spontaneous hematological recovery nor salvage HSCT. The other 7 patients received salvage HSCT for PGF. The median interval from graft failure to salvage HSCT was 26 days (range, 22–50 days). Of 7 patients with PGF, one patient received BMT from same sibling donor of prior HSCT, other three patients were rescued by BMT from haplo-identical parents, the other 4 patients received u-CBT; all of them were prepared by non-myeloablative conditioning. After salvage HSCT, 6 patients achieved engraftment (median, day + 22; range, day + 8 to + 26); the other patient with ALL who received u-CBT as salvage HSCT developed PGF again, and received haplo-identical BMT from his father at day + 30 after second HSCT. Six of eight patients with PGF died of hepatic VOD ($n = 2$), acute graft versus host disease (GVHD, $n = 1$), chronic GVHD ($n = 1$), and relapse ($n = 2$); the other two patients survived 29 months and 91 months after salvage SCT, respectively. The overall survival at 2 years after PGF for patients with malignant disease was 38% (95%CI, 9-67%). High risk disease status, third or more complete remission or non-remission at first SCT, was the only poor prognosis factor of survival for patients with PGF (Hazard ratio, 11; 95% confidence interval, 1.2 - 102; $P = 0.036$).

Conclusion: Our findings suggest that efforts toward early diagnosis of PGF could lead to immediate salvage HSCT at an

appropriate timing and that both u-CBT and haplo-identical BMT were comparable options as salvage SCT for PGF.
Disclosure of Interest: None declared.

P599

Longitudinal decline of body-mass-index (BMI) and low serum albumin correlate with incidence of sepsis and non-relapse mortality (NRM) beyond day +110 after allogeneic hematopoietic stem cell transplantation (SCT)

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Introduction: Infections with ensuing sepsis contribute to the high incidence of NRM post-SCT. They not only occur during the acute post-SCT phase but also later, beyond 3 months after SCT. There are diverse known risk factors for late NRM, but little data are available for nutrition-related parameters like BMI and albumin. As nutrition contributes to the immune-status we hypothesized that such parameters correlate with the incidence of sepsis and NRM beyond day (d) +110 post-SCT.
Materials (or patients) and methods: In a retrospective study we analyzed data from start of conditioning (pre-SCT) until one year post-SCT from 128 patients who underwent allogeneic SCT at our center. For longitudinal analysis this period was divided into two intervals: pre-SCT until d +110 (IN1) and from d +110 until d +365 (IN2).
Results: For pre-SCT data only moderate/severe albumin deficiency (OR 4.6, CI 1.5-13.9, $P = .006$) but not BMI and not total serum protein deficiency correlated with NRM beyond d +110. While most patients experienced a BMI reduction in IN1 (88%), no obvious trend was seen in IN2 (45% reduced, 55% stable/increased). In uni- and multifactorial analysis the incidence of sepsis beyond d +110 correlated with a continuous BMI reduction in IN1 and IN2 (OR 6.1, CI 1.2-32.4, $P = .03$) as well as with a moderate/severe albumin deficiency on d +110 (OR 6.8, CI 1.4-31.8, $P = .02$) and a deficiency in total serum protein on d +110 (OR 6.0, CI 1.2-29.3, $P = .03$). Survival analysis revealed a correlation for NRM beyond d +110 with a BMI reduction in IN2 (Log Rank $P = P = .04$), with a continuous reduction of BMI in IN1 and IN2 (Log Rank $P = .04$) as well as with a moderate/severe albumin deficiency on d +110 (Log Rank $P = .004$) and deficiency in total serum protein on d +110 (Log Rank $P = .03$). Uni- and multifactorial regression models showed a higher risk for NRM beyond d +110 in patients with a BMI reduction in IN2 (OR 5.1, CI 0.9-26.6, $P = .05$), in patients with a continuous BMI reduction in IN1 and IN2 (OR 8.2, CI 0.9-74.4, $P = .06$) and in patients with a moderate/severe albumin deficiency on d +110 (OR 8.4, CI 1.5-48.1, $P = .02$). The association of deficiency in total serum protein on d +110 with higher risk for NRM beyond d +110 was not significant in multifactorial analysis (OR 4.8, CI 1.0-23.5, $P = .06$).
Conclusion: We conclude that continuous BMI reduction beyond d +110 and pronounced deficiency of albumin on d +110, and to a lesser degree total serum protein on d +110 represent a prolonged poor nutritional status and correlate with the occurrence of sepsis and NRM beyond d +110 after allogeneic SCT. These findings highlight the need for further studies on the impact of nutritional status and on possible interventions for the long-term outcome of allogeneic SCT.
Disclosure of Interest: None declared.

P600

Response to immunization with hepatitis B, varicella, measles, mumps and rubella in pediatric patients after hematopoietic stem cell transplantation

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Introduction: There are a several guidelines published on immunization post hematopoietic stem cell transplantation (HSCT), but very few publications on their immune responses after.
Materials (or patients) and methods: A retrospective review of pediatric HSCT patients who completed at least 2 years of post HSCT follow up at our center.
Results: There were 51 allogeneic HSCT patients reviewed who were followed up for late effects from Oct 2001 to Feb 2012. Only 39 out of 51 had data for immunization of hepatitis B, measles, mumps and rubella (MMR) and varicella (VZV). All 39 received 6-in-1 (infanrix-hexa) series at a minimum of 12 months post HSCT; 26 (66%) mounted positive Hepatitis B after 1 series. VZV was administered as MMR-V or VZV to 28 patients at a minimum of 2 years and off immunosuppression; 10 did not receive VZV due to positive serology pre-immunization (some developed chicken pox/herpes zoster); 1 was still on immunosuppression at 2 years. Fifteen patients received MMR-V (22% positive in those with repeat serology); 13 patients received VZV (38% positive); Fourteen (50% received second doses of either MMRV#2 (75% pos) or VZV#2 (60% pos). Four (13%) received a third dose of VZV/MMR-V before getting a positive response. Of the 15 patients who received MMR-V, positive responses were: measles (92%), mumps (50%), and rubella (100%). Of the 21 patients who received MMR, positive responses were: measles (78%), mumps (66%), rubella (88%); 1 patient received 2 doses of MMR and still negative for measles after; At least 3 patients who received either MMR or MMR-V remains negative for mumps after 2 doses. We also noted a decline in seropositive titers in some patients after repeating a few years after immunization, with some becoming negative even.
Conclusion: Measuring responses to immunization is necessary in the follow up of patients post HSCT. A significant number fail to achieve immune responses after one series of immunization. It is worthwhile to correlate the CD4 numbers in these patients.
Disclosure of Interest: None declared.

P601

Evidence of defibrotide internalization and its endothelial protective effect in hepatic endothelial *in vitro* model

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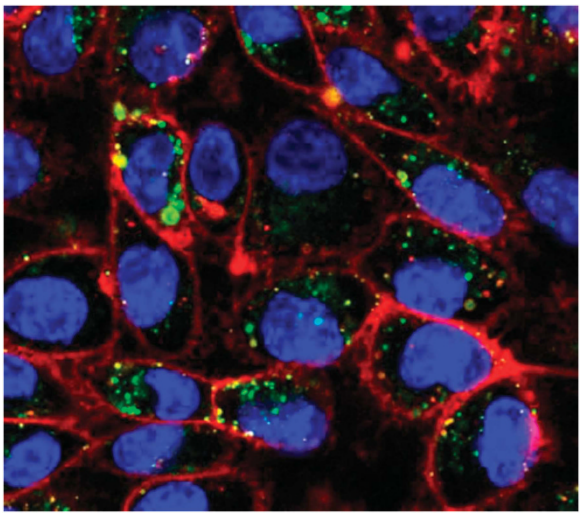
Introduction: Hematopoietic Stem Cell Transplantation (HSCT) is associated with several early and late life-threatening complications, some of which are suspicious to have an endothelial dysfunction origin. Defibrotide (DF) interferes with several steps of the coagulation-inflammation cycle and has a potential role as endothelial protective agent. Last October (2013), DF received EMA authorization for its use in the treatment of severe hepatic veno-occlusive disease (VOD) after HSCT. Since endothelial dysfunction has been implied in the development of VOD, among other HSCT associated complications, we decided to explore the mechanisms of action of DF in a hepatic endothelial in vitro model. To investigate the interaction of DF with a hepatic endothelial cell line from human origin (SK-HEP-1) and the mechanisms involved in its traffic.
Materials (or patients) and methods: SK-HEP-1 cells were exposed to DF (4 μg/ml), previously labelled with ULYSIS® Nucleic Acid Labeling Kits, for up to 24 hours. Using inhibitory assays and flow cytometry techniques (Navios - Beckman Coulter, Inc.) along with confocal microscopy (Leica TCS SP5), we have explored: 1) DF internalization, and dose-response kinetics, and 2) different pathways of endocytosis.
Results: Flow cytometry assays revealed concentration, temperature and time dependent up-take of DF by Sk-HEP-1 cells. Moreover, inhibitory assays indicate that entrance of DF into endothelial cells occurs primarily through macropinocytosis, and that this mechanism seems to be highly dependent on actin assembly followed by endosome traffic. Confocal microscopy



allowed visualization of significant interaction of DF with endothelial cell membranes followed by internalization and redistribution to the cytoplasm. DF did not reach the cell nucleus even after 24 h of exposure. (Image: Defibrotide (in green) internalization by Sk-Hep1 cells (membranes and nuclei labelled in red and blue colour, respectively)

Conclusion: Our studies show that DF interacts with endothelial cells membranes, becoming internalized and redistributed into endothelial cell compartments without evidence in the nucleus. Our findings may contribute to a better understanding of the precise mechanisms of action of DF as a therapeutic and potential preventive agent on the endothelial damage underlying different pathological situations.

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P602
Low dose of Deferasirox treatment in patients actually free of transfusion who present iron overload after bone marrow transplantation

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Introduction: Recipients of hematopoietic stem cell transplantation (HSCT) frequently have iron overload (IO) resulting from chronic transfusion therapy for haematological disorders. The commonest cause is an improper or complete absence of iron chelation therapy before and during the treatments which proceed transplantation. There is evidence that IO may affect outcome of allogeneic HSCT in term of higher transplant-related mortality and late complications, but the exact role of preventive iron chelation therapy with deferasirox (DFX) in transplanted patients (pts) is not yet known.

Materials (or patients) and methods: In order to evaluate the efficacy of DFX after bone marrow transplantation, we considered a group of pts who underwent HSCT with a previous high iron uptake (more than 20 blood transfusions), who showed a ferritin level upper of 1000 ng/ml, a normal renal function, and a favourable post-transplant outcome (complete remission and absence of significant complications, included severe active graft versus host disease). They must to be absolutely free of transfusion dependence too. With the same criteria, we included also pts with a lower ferritin level, but with a histologic demonstration of liver IO. Considering the contemporary absence of iron intake, we treated them with low-dose of DFX (5-10 mg/Kg/day). The treatment started in a period between 3 and 12 months after HSCT, in absence of significant transplant complication, and prosecuted until a reduction of serum ferritin value lower than 1000 ng/ml. Suspension of treatment has been considered in case of DFX related side effects.

Results: We overall treated 18 pts (2 of them for liver IO). Median initial ferritin level was 2200 ng/ml (1000-7482 ng/ml). Median duration of treatment was 10 months (1-22 months). 10 pts completed the treatment obtaining a median final ferritin value of 850 ng/dl. 2 pts are still ongoing and 6 pts stopped early the treatment for toxicity (4) or disease relapse (2). DFX toxicity has been observed in 5 pts with renal impairment (2), diarrhea (2) and liver toxicity (1), only one of them restarted and prosecuted the treatment. All the pts treated for at least 3 months experienced a reduction in ferritin burden. It is interest to note the improvement in the haemoglobin (Hb) level observed in some pts: 4 experienced a Hb increase upper than 3 g/dl and 2 pts between 1 and 3 g/dl. Maybe they can be considered as "haematological responders". On the other hand 12 pts did not show significant Hb improvement.

Conclusion: These preliminary data tell us that low dosage of DFX can be considered and efficient option in transplanted pts who present IO in order to reduce ferritin burden. Although some pts need to stop the treatment for toxicity, the other ones who prosecute the care, present a good result in terms of ferritin level reduction and, in some cases, of haemoglobin improvement. Largest studies need to confirm this suggestion.

Disclosure of Interest: None declared.

P603
Circulating HCMV-miR-US25-1-5p is risk factor and potential regulatory hub for late-onset hemorrhagic cystitis following allogeneic hematopoietic stem cell transplantation

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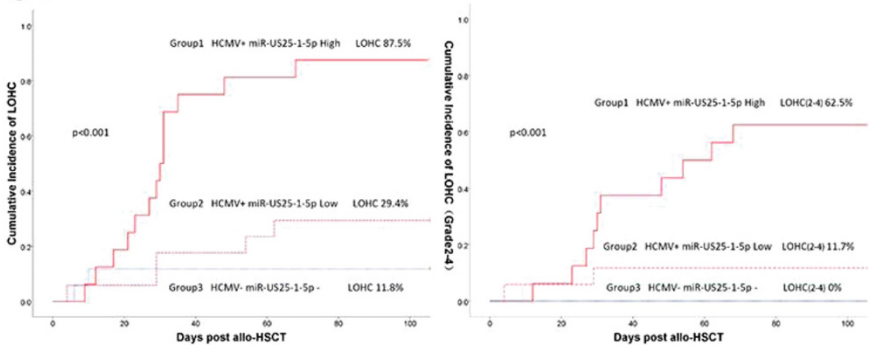
Introduction: Late-onset hemorrhagic cystitis (LOHC) is a common complication following allo-HSCT. Our previous studies supported an important role of human cytomegalovirus (HCMV) for development of LOHC[1]. Recently viral microRNAs (miRNAs) emerged as critical regulators of host genes by conserved networks of host mRNA target sites. This study is to identify potential roles of HCMV miRNAs in prediction and pathogenesis of LOHC.

Materials (or patients) and methods: Quantitative real-time polymerase chain reaction (qRT-PCR) with SYBR-green were employed to screen differentially expressed HCMV pre-miRNAs in paired plasma samples from 5 patients with HCMV-viremia associated LOHC and after complete response. Then candidate HCMV mature miRNAs, together with HCMV and BKV DNA loads were further validated in 50 consecutive patients for systemic monitoring and 71 patients for symptomatic follow-up of LOHC after allo-HSCT. Plasma were collected before conditioning, once per week between infusion of grafts (d0) and d + 30, once per two weeks before d + 90), and twice per week during LOHC. The absolute copies of miRNAs were normalized by the standard curve in TaqMan qRT-PCR. The candidate miRNAs were finally introduced into co-regulatory network analysis and targets validation.

Results: HCMV-miR-US25-1-5p (US25-1-5p) was chosen as candidate miRNAs from all kinds of HCMV encoding pre-miRs ($n = 15$, illustrated in clusters: UL22A; UL36; UL59/69/70; UL112; UL148D; US4/US5-1/US5-2; US22-1/US25-1/US25-2/US29-1/US33-1). In consecutive cohort, 21 patients developed LOHC with a median day of 29 (4–64) days. Patients were

[P603]

Figure-1



divided into 3 groups before LOHC onsets: HCMV viremia (HCMV+) with high US25-1-5p expression $>3\times10^3$;$n=16$), HCMV+ with Low US25-1-5p ($<3\times10^3$;$n=17$) without HCMV viremia(HCMV-). Cumulative incidence of LOHC (1-4 or 2-4) was higher in patients of group 1 compared with the other two groups post-HSCT ($P<0.001$, $P<0.001$, Figure-1). Multivariate analyses showed that High US25-1-5p expression was independent risk factor for the development of grade 2-4 LOHC (HR8.533, 95CI%: 1.708-42.631, $P=0.009$). Patients with low US25-1-5p presented better overall response rate to antiviral treatment (81.8% vs.40%, $P<0.001$), while the other group response better to corticosteroids. In co-regulatory network by bioinformatics and in vitro validation, US25-1-5p, ROS, NLRP3 and NF-KB were found as hubs of feed forward loop in pyroptosis of bladder epithelium and smooth muscle. Up-regulated HCMV-miR-US25-1-5p may strengthen the pro-inflammatory signals by activating NLRP3 inflammasome and NF-KB pathway and lead to pyroptic cell lysis.

Conclusion: Our study suggest that circulating HCMV-miR-US25-1-5p is independent risk factor for development of LOHC. US25-1-5p might have better prognostic value than HCMV viremia and predict the response to corticosteroid in LOHC, which could contribute to the regulatory roles of US25-1-5p in the uncontrolled inflammatory feedback and promotion of pyroptosis in LOHC pathogenesis.

References: [1]Han, T. T., Xu, L. P., Liu, D. H., Liu, K. Y., Fu, H. X., Zhao, X. Y., Zhao, X. S. and Huang, X. J., Cytomegalovirus is a potential risk factor for late-onset hemorrhagic cystitis following allogeneic hematopoietic stem cell transplantation. Am J Hematol 2014. 89: 55-61.

Disclosure of Interest: None declared.

P604

Quality of life (QoL) assessment in patients allografted for poor-risk chronic lymphocytic leukemia (CLL)

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Introduction: Little is known about quality of life (QoL) of survivors of allogeneic hematopoietic stem cell transplantation (alloHCT) for CLL.

Materials (or patients) and methods: In a single center cross-sectional analysis, QoL and employment status were assessed in patients allografted for poor-risk CLL. Eligible were all living patients who had undergone alloHCT for CLL at our institution between June 2005 and April 2013. Eligible patients ($n=53$) were asked to complete the FACT-BMT questionnaire, a validated measure of QoL in allografted patients, considering five dimensions of QoL: physical well-being (PWB), social (SWB), emotional (EWB) and functional well-being (FWB) as well as transplant-specific aspects (bone marrow transplantation subscale, BMTS). An extra item was added that surveyed the employment status.

Results: 40 of 53 questionnaires returned and were evaluable. Of the 40 patients at a median age of 59 years (43-72), 28 (70%) were male. With a median of 3 (1-8) previous therapies, the remission status before alloHCT was CR in 3 patients, PR in 32 and The median time from alloHCT to QoL assessment was 50 months (9-98). Of 30 patients that reported about employment status, 15 (50%) had resumed a fulltime job after alloHCT. The median time from alloHCT to fulltime employment was 10 months (2-33).

The median reported subscale scores were PWB 25/28, SWB 24/28, EWB 20/24, FWB 21/28, BMTS 31/40, resulting in a median FACT-BMT total score of 122/148 (range 87-144/148). This implies that exactly half of the patients reported a good to excellent aggregated QoL and the other half an intermediate to good aggregated QoL at the time of assessment.

The total score tended to be lower in patients that were not employed after alloHCT, not MRD negative 12 months after alloHCT or that had progressive disease, in patients transplanted from a foreign donor or from a mismatch donor, in patients with a cGvHD history and still on systemic immunosuppression 12 months after alloHCT. However, none of the scoring differences reached statistical significance with the patient number studied here. In patients that were fulltime employed after alloHCT, the functional well-being (FWB) subscale score was significantly higher ($P=0.0265$). In patients aged >55y at referral, the emotional well-being (EWB) was significantly higher ($P=0.0331$). Age, time to immunosuppression tapering and the number of previous therapies did not significantly affect the scoring.

Conclusion: Although limited by its small sample size and its cross-sectional design, this study provides preliminary evidence that QoL is rarely perceived as strongly reduced but largely (half and half) scored as intermediate to good and good to excellent, respectively. Notably, a high proportion of patients resumed fulltime work activity after alloHCT, which significantly affected functional well-being. These results need to be corroborated by larger, ideally prospective studies.

Disclosure of Interest: None declared.

P605

Association Between High Uric Acid Levels and Hepatic Veno-Occlusive Disease in HCST

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Introduction: Hepatic veno-occlusive disease (VOD) is one of the most important early life-threatening complications of hematopoietic stem cell transplantation (HSCT). Sinusoidal endothelial damage is initiated by several factors including conditioning, cytokines released by damaged tissues, drugs, endotoxins and immunological reactions. Uric acid (UA) is a known danger signal released from injured cells and

induces expansion of alloreactive T cells but its role in VOD is unclear.
Materials (or patients) and methods: Two hundred and fifty six children (median age 7 years, range: 0,1-19; M/F:173/83) transplanted at Pediatric BMT Unit of Hacettepe University between 2000 and 2014 were included in this retrospective study. Serum UA levels were recorded prior to the initiation of conditioning (day -9) and at the time of HSCT (day 0) were analyzed. The association between serum UA levels and development of hepatic VOD was assessed.
Results: Most patients undergoing HSCT from matched-related donors (89%) received myeloablative conditioning (73%) and transplanted for non-malignant diseases (74%). Cyclosporine and methotrexate were administered for GVHD prophylaxis. Bone marrow was the main stem cell source (73%). Forty five patients who developed VOD had higher median serum UA levels at day -9 compared to those who did not develop VOD (3.51 mg/ml vs 3.0 mg/ml, $P = 0.019$) and this difference remained significant at the day of HSCT (3.05 mg/ml vs 2.69 mg/ml $P = 0.032$). While the serum UA levels of the patients who did not developed VOD decreased significantly at the day of HSCT (3.0 mg/ml vs 2.69 mg/ml $P = 0.033$), there was no significant change in serum UA levels of patients who developed VOD (3.51 mg/ml vs 3.05 mg/ml $P = 0.06$). The development of VOD was associated with a higher UA level at day-9 ($P = 0.002$). When subjected to multivariate analysis only UA level at day-9 remained a significant predictor of VOD ($P = 0.005$) among other risk factors such as donor type and conditioning.
Conclusion: Our results suggest that high serum UA levels during pre-tranplantation period might predict the development of hepatic VOD in patients undergoing allogeneic HSCT. Confirmation with prospective studies and investigation of underlying mechanisms are needed.
Disclosure of Interest: None declared.

P606
Association of Psycho social Outcomes with Familial Financial Hardship after Hematopoietic Cell Transplantation

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Introduction: Increased financial burden from costs of cancer care is known to be associated with poor quality of life among cancer survivors. Hematopoietic cell transplantation (HCT) is a potentially curative treatment modality for hematological disorders but may result in extensive morbidity and death. While preliminary data suggest that financial hardship is prevalent among HCT patients, little is known about its impact on their psychosocial health.
Materials (or patients) and methods: A 43-item questionnaire regarding financial concerns, household income, employment status and insurance was mailed to adult patients approximately six months post-allogeneic or autologous HCT at three sites (Dana-Farber Cancer Institute [DFCI], Mayo Clinic Arizona [MCA], and Roswell Park Cancer Institute [RPCI]). The questionnaire included the Perceived Stress Scale (PSS-4; scores range from 0 to 16 with higher scores indicating higher stress) and a single seven-point linear analogue self-assessment scale to document overall quality of life (QOL; higher scores indicate better QOL). Prospective HCT-related clinical outcomes associated with financial hardship will be assessed at one year post-HCT. In this analysis, we report preliminary data regarding the cross-sectional association of financial hardship with perceived stress and QOL at six months post-HCT.
Results: As of this analysis, 240 surveys have been received: 188/280 at DFCI (response rate [RR] = 67%); 33/72 from MCA (RR = 46%), and 19/42 from RPCI (RR = 45%). Mean age of respondents was 58 years, and they were a median of 183 days post-HCT; additional baseline characteristics are shown in the Table. Overall, 50% of respondents were dissatisfied with their present financial situation, 43% had difficulty paying monthly bills and 47% reported a decline in their income due to HCT. In addition, 19% had to borrow money to pay bills, and 44% reduced spending on daily necessities as a result of financial difficulties. We defined substantial financial hardship as not having enough money at the end of the month, which was reported by 21% of the respondents. The mean PSS-4 score was 5.1 [4.7 - 5.6], and the mean QOL score was 5.1 [4.9 - 5.3]. In multivariable models controlling for gender, age, transplant type and transplant center, the presence of substantial financial hardship was associated with higher than average perceived stress (OR 2.68 [1.28 - 5.60] $P = 0.008$). In multivariable models including the same covariates, substantial financial hardship predicted lower than average QOL (OR 0.37 [0.19 - 0.74]; $P = 0.005$).

Table: Baseline characteristics

Characteristic	N	%
Allogeneic HCT	111	46.3
Female	96	40.0
White Race	221	92.1
Employed (Working or Taking Leave)	109	45.6
Education (Bachelor/ Graduate Degree or higher)	119	49.8
Monthly Income		
Less than $3,000	65	28.0
$3,000 to $6,999	105	45.3
f$7,000 and over	62	26.7

Conclusion: In our large multi-site cohort of HCT patients, substantial financial hardship at six months was significantly associated with higher perceived stress and lower quality of life. These data highlight the impact of financial difficulty after HCT on patients' overall well-being. Future efforts should focus on identifying vulnerable patients to connect them to appropriate resources.
Disclosure of Interest: None declared.

P607
Long-Term Side Effects of Hematopoietic Stem Cell Transplantation: A Single Center Experience

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Introduction: Hematopoietic stem cell transplantation (HSCT) provides effective therapy for patients with hematopoietic, immunologic, metabolic and other disorders. Both the annual number of HSCT procedures has increased dramatically and the number of diseases for which HSCT is considered appropriate has expanded over the years. These improved outcomes have resulted in many long-term survivors who are experiencing substantial long-term morbidities.
Materials (or patients) and methods: Fifty-one patients who underwent allogeneic HSCT between 2001-2014 and survived for at least two years were enrolled in this study. Patients were followed-up for complications or late side effects after HSCT regularly.
Results: Mean follow-up period after HSCT was 68.3 ± 31.4 months (min. = 24-max. = 141), patients' age average was 9.3 ± 4.9 years (min. = 0,5-max. = 17), 56.9% of them were male, and 92.2% ($n = 47$) of donors were match family donors. Most of the long-term side effects were endocrine complications with 17.6% ($n = 9$), short stature and hypogonadism were notable with 7.8% ($n = 4$) individually. Ocular long term side effects were increased in haploidentical and match unrelated donor (MUD) transplantations ($P = 0.002$). Furthermore ocular late side effects were correlated with age, ($P = 0.003$) serum ferritin levels, ($P = 0.004$) and mean corpuscular volume ($P = 0.003$) positively. We found that chronic graft versus host disease (GVHD) rate was increased with MUD transplantation

[P607]

Long-Term Side Effects	n	%
Respiratory	5	9.8
Cardiac and vascular	1	2
Endocrine	9	17.6
Ocular	8	15.7
Liver	4	7.8
Renal and genitourinary	3	5.9
Immune system	7	13.7
Oral	3	5.9
Skeletal	6	11,8
Nervous system	2	3.9
Mucocutaneous	6	1.8
Second cancers	0	0

($P = 0.003$) and acute liver GVHD ($P = 0.002$). Osteoporosis frequency was positively correlated with follow-up time ($P = 0.002$) and found much more in malign disorders than benign ones ($P = 0.002$). We couldn't find any difference among conditioning regimens as regard of late side effects. However, osteoporosis was rarely found in conditioning regimens included cyclophosphamide ($P = 0.004$). Long-term side effect risk was higher in patients underwent HSCT in advanced age than younger age ($P = 0.003$)

Conclusion: Cyclophosphamide based conditioning regimens had low osteoporosis risk. The main reason of osteoporosis in malign pathologies may be associated with previous cancer treatment. Haploidentical and match unrelated donor (MUD) transplantation increase long-term side effects. Regular monitoring is important to detect and treat these side effects.

Disclosure of Interest: None declared.

P608

A Phase 1, Open-label Study to Investigate the Effect of Hemodialysis on Plasma Defibrotide Pharmacokinetics in End-stage Renal Disease Patients

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Introduction: Hepatic veno-occlusive disease (VOD; also called sinusoidal obstruction syndrome) is a potentially fatal complication of *hematopoietic* stem cell transplantation (HSCT). Severe VOD (sVOD) is usually characterized by multi-organ failure (MOF; eg, pulmonary and/or renal failure), and associated with >80% mortality. sVOD may develop in a substantial number of high-risk pts. Defibrotide (DF) protects endothelial cells from transplant-induced damage and restores the thrombotic-fibrinolytic balance. DF is *approved in the European Union for the treatment of severe hepatic VOD in HSCT pts.*

Materials (or patients) and methods: This open-label study assessed the effect of hemodialysis on the pharmacokinetics (PK) of DF in dialysis-dependent, end-stage renal disease (ESRD) pts. DF safety and tolerability was also assessed. Pts were men and women 18 to 80 years of age, with estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m^2 and on dialysis. Each pt received 2 doses of DF 6.25 mg/kg administered by IV infusion over 2 hours ($\pm$ 10 minutes): 1 dose on a nondialysis day (Day 1) and 1 on a dialysis day (Day 4). Dialysis began 1 hour after the start of DF infusion and occurred over 4 hours. Key PK parameters included area under the plasma concentration-time curve (AUC) from start of infusion (time 0 hour) to time of the last quantifiable plasma concentration following dosing ($AUC_{0\text{-}t}$) and extrapolated to infinity ($AUC_{0\text{-}\infty}$), as well as maximum observed plasma concentration (C_{max}), time of C_{max} (t_{max}), apparent terminal phase half-life ($t_{1/2}$), systemic plasma clearance (CL), and volume of distribution at steady state (V_{ss}). A linear mixed effects model was performed on natural log-transformed values of DF $AUC_{0\text{-}\infty}$ and C_{max} on nondialysis (Day 1) and dialysis days (Day 4). Point estimates and 90% confidence intervals (CIs) for log scale differences were exponentiated for estimated ratios of geometric least-square (LS) means on the original scale, with Day 1 as the reference treatment.

Results: The study included 6 pts. The plasma DF for key PK parameters following DF dose on Days 1 and 4 are shown in the Table.

Table

	Day 1	*Day 4*
$AUC_{0\text{-}t}$, (μg · h/mL) mean (CV%)	102 (40.0)	111 (39.9)
$AUC_{0\text{-}\infty}$, (μg · h/mL) mean (CV%)	103 (40.3)	114 (40.6)
C_{max} (μg/mL), mean (CV%)	45.1 (35.1)	50.1 (38.1)
$t_{1/2}$ (h), mean (CV%)	0.712 (21.9)	0.967 (17.6)
CL (L/h), mean (CV%)	5.87 (24.9)	5.38 (26.1)
V_{ss} (L), mean (CV%)	6.34 (33.2)	6.90 (28.4)
t_{max} (h), median (min, max)	1.90 (1.50, 1.95)	1.78 (1.75, 1.95)

The % ratio of LS means (90% CI) following DF dose on Days 1 and 4, respectively, were: C_{max}, 109.71 (97.23, 123.78); $AUC_{0\text{-}t}$, 108.39 (97.85, 120.07); and $AUC_{0\text{-}\infty}$, 109.98 (99.39, 121.70).
One pt had a nonserious treatment-emergent adverse event (AE; vomiting, mild severity) that was judged possibly related to study treatment. No other adverse safety/tolerability findings were reported.
Conclusion: The percent ratio of Day 4 (dialysis day) to Day 1 (nondialysis day) LS means and 90% CIs of $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, and C_{max} were within the 80% to 125% range. Therefore, hemodialysis did not significantly affect exposure to DF, and had no notable effect on plasma DF clearance in dialysis-dependent ESRD pts. AEs in ESRD pts on hemodialysis were consistent with previous studies.
Support: Jazz Pharmaceuticals
Disclosure of Interest: P. Tocchetti Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., M. Ballabio Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., E. Tudone Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., J.-F. Marier Employee of: Pharsight, a Cetara Company, Conflict with: employee of research organization that provided services to Jazz Pharmaceuticals, T. C. Marbury Employee of: Orlando Clinical Research Center, Conflict with: employee of research organization that provided services to Jazz Pharmaceuticals, K. Zomorodi Employee of: Jazz Pharmaceuticals, Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., M. Eller Employee of: Jazz Pharmaceuticals, Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc.

P609

A Phase 1, Open-label Study of Defibrotide Pharmacokinetics in Severe/End-stage Renal Disease Patients not on Dialysis Compared with Healthy Matching Subjects

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Introduction: Hepatic veno-occlusive disease (VOD; also called sinusoidal obstruction syndrome) is a potentially fatal complication of hematopoietic stem cell transplantation (HSCT). Severe VOD may develop in a substantial number of high-risk pts and is often unpredictable. It is typically characterized by multi-organ failure (MOF; eg, pulmonary and/or renal) and ultimately results in an $>80\%$ mortality rate. Defibrotide (DF) protects endothelial cells from transplant-induced damage and restores the thrombotic-fibrinolytic balance. It is approved in the European Union for the treatment of severe hepatic VOD in HSCT pts.
Materials (or patients) and methods: This phase 1, open-label study compared the plasma pharmacokinetic (PK) profile of DF in pts with severe or end-stage renal disease (ESRD) and healthy matching subjects. Safety and tolerability were also assessed. The severe/ESRD pts (Cohort 1) were men and women aged 18-80 years with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 and not on dialysis. The healthy subjects (Cohort 2) had normal renal function (eGFR ≥ 90 mL/min/1.73 m^2 for subjects aged 18-59 years; ≥ 80 mL/min/1.73 m^2 for subjects aged 60-80 years) and were matched with Cohort 1 pts in terms of age (± 10 years), body mass index ($\pm 20\%$), gender, and race. Both cohorts were treated with DF 25 mg/kg as 4 divided doses (6.25 mg/kg/dose) administered by 2-hour IV infusion every 6 hours for 24 hours. PK parameters included area under the plasma concentration-time curve (AUC) from start of infusion (time 0) to time of the last quantifiable plasma concentration following dosing ($AUC_{0\text{-}t}$), to the 6-hour length of the dosing interval (AUC_{τ}), and extrapolated to infinity ($AUC_{0\text{-}\infty}$). Maximum observed plasma concentration (C_{max}) was also determined. A linear mixed effects model was used to compare log-transformed PK parameters in Cohort 1 and Cohort 2 (control group) after dose 1 (Day 1) and dose 4 (Day 2). Point estimates and 90% confidence intervals (CIs) were exponentiated to obtain estimates for ratios of geometric least-squares (LS) means on the original scale.
Results: Six severe/ESRD pts (Cohort 1) and 6 healthy matching subjects (Cohort 2) were included. Key PK results are shown in the Table. Half-life in Cohorts 1 and 2 were 0.725 h and 0.562 h after dose 1, and 0.498 h and 0.217 h after dose 4, respectively.

Table.

	Geometric LS means		*% Ratio of LS means (90% CI)*
	ESRD	*Healthy*	
Dose 1			
C_{max}	53.6 μg/mL	39.6 μg/mL	135.37 (105.06, 174.42)
$AUC_{0\text{-}t}$	113.4 μg · h/mL	74.5 μg · h/mL	152.18 (117.60, 196.94)
$AUC_{0\text{-}\infty}$	114.6 μg · h/mL	74.9 μg · h/mL	153.01 (117.70, 198.91)
Dose 4			
C_{max}	52.6 μg/mL	38.0 μg/mL	138.34 (106.05, 180.46)
$AUC_{0\text{-}t}$	108.9 μg · h/mL	68.3 μg · h/mL	159.55 (118.15, 215.47)
AUC_{τ}	109.0 μg · h/mL	68.4 μg · h/mL	159.36 (118.11, 215.05)

No treatment-emergent adverse events (AEs) were reported.
Conclusion: DF exposure was higher in severe/ESRD, nondialysis pts than in healthy matching controls after single and multiple doses. The PK exposure parameters after multiple doses were within 5% $>$ 8% of exposure parameters observed after the first dose of DF in both cohorts. This is consistent with the short half-life of DF compared to the dosing interval. Therefore, there was no accumulation after repeated dosing in either severe/ESRD pts or healthy controls. AEs in both renal impaired and control cohorts were consistent with previous studies.
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Disclosure of Interest: P. Tocchetti Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., M. Ballabio Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., E. Tudone Employee of: Gentium S.p.A., Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., J.-F. Marier Employee of: Pharsight, a Cetara Company, Conflict with: employee of research organization that provided services to Jazz Pharmaceuticals, T. C. Marbury Employee of: Orlando Clinical Research Center, Conflict with: employee of research organization that provided services to Jazz Pharmaceuticals, K. Zomorodi Employee of: Jazz Pharmaceuticals, Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc., M. Eller Employee of: Jazz Pharmaceuticals, Conflict with: received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals plc.

P610

Retrospetive study about donor lymphocyte infusions for relapse after allogeneic hematopoietic transplantation in hematological malignancies: analysis of risk factors and outcomes after twenty years of follow up

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Introduction: Relapse after hematopoietic transplantation may be treated with donor lymphocyte infusions (DLI). Most

common side effect is graft-versus-host disease (GVHD). The goal of this study is to evaluate global survival after DLI and risk factors for mortality and GVHD.
Materials (or patients) and methods: We included 36 patients from Hospital Reina Sofia that received DLI after relapse from alogeneic transplantation. Data gathered from regarding original bone marrow transplant, relapse, DLI, response and complications of DLI.
Results: 34 patients remain in the study, with 61 DLI (median followed time: 90.8 months). Prophylactic and preventive DLI had an overall survival (OS) is 100% (for patients with mixed chimerism or high risk of relapse in complete remission of the hematological malignancy). In case of therapeutic DLI (after clinical relapse) the OS 3 years was 75% ($P = 0.0128$). Multivariate analysis for mortality risk factors showed that patients with transplants made before 1997 had more risk; while patients with chronic myelogenous leukemia (CML) and those with complete remissions of disease had less risk of death ($P = 0.0006$; $P = 0.0001$). Multivariate analysis for GVHD's risk factors showed less risk in lower than $1x10^8$/Kg CD3 cell doses and more than 3 months separated DLI ($P = 0.045$; $P = 0.002$).
Conclusion: Fewer complete remissions and higher mortality was observed in hematological malignancies different from CML and in advanced states. GVHD is common after DLI and is less observed with lower doses of CD3 and more time between doses.
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P611
Serum citrulline level in evaluation of gastrointestinal toxicity after high-dose chemotherapy

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Introduction: Over 80% of stem cell transplant patients develop a mucous membrane reaction. Recent years have seen the emergence of reliable clinical data on the correlation of plasma citrulline levels with the severity of intestinal mucosal damage in hematopoietic stem cell transplantation recipients. An attempt was made to assess the usefulness of measuring plasma citrulline levels to evaluate damage to intestinal mucosa in routine clinical practice.
Materials (or patients) and methods: The study group included 50 consecutive patients seeking treatment at the Bone Marrow Transplant Center, Military Institute of Medicine (WIM) between November 2007 and February 2010. Patients who met the inclusion criteria and signed the consent form proceeded to undergo high-dose chemotherapy with hematopoietic stem cell transplantation. Due to the variety of the underlying diseases and transplant indications the conditioning regimens that were used were not homogeneous. During the study, no patients received new medicinal products, and treatment was conducted according to well established standards. According to study protocol, every day during hospitalization at the Bone Marrow Transplant Center, WIM, each patient had their medical history taken and a physical examination was conducted. The severity of gastrointestinal symptoms was assessed everyday. Every patient had a blood sample collected 6 times for measuring citrulline and CRP levels.
Results: Median age of the 50 patients included in the study was 46.5 years (range 20-64). Eighty-four percent of the study group developed gastrointestinal side effects. The severity 75 of gastrointestinal symptoms was evaluated using a 16-point cumulative intestinal toxicity scale. The severity increased significantly, peaking at Day 7 ($P<0.0001$) following transplantation (mean 3.9; 95% CI 3.1–4.7; range 0-14). In the group of patients in the present study, the average initial citrulline level, before chemotherapy was lower 24.8 μm/l (95% CI 22.3–27.2, range 10.2–50.8) in comparison to healthy population (40 ± 10 μm/l). The trough citrulline level was reached in the first week post-transplantation. The mean citrulline level was significantly lower ($P = 0.0001$) at Day 7 (mean concentration 8.5 μm; 95% CI 7.9–9.9; range 2.4–29.6) in comparison to baseline. A CRP kinetics analysis conducted up to Day 21 showed the highest increase of CRP on Day 7 ($P < 0.0001$). Day 7 CRP levels correlated with the severity of gastrointestinal symptoms ($r = 0.46$; $P = 0.0009$) and negatively correlates with changes in CRP levels ($R = -0.58$; $P < 0{,}0001$).
Conclusion: Citrulline levels and their variability during the transplant procedure are a biomarker for the level of intestinal mucosa damage. The change in serum citrulline levels negatively correlates with the severity of intestinal damage and changes in CRP levels. A high baseline citrulline level is a poor prognostic marker for developing gastrointestinal symptoms. Oral mucositis and distal gastrointestinal mucositis differ in the time of occurrence and clinical presentation. Therefore, they should be considered to be separate entities. Our study demonstrated no correlation between hematopoietic system regeneration or febrile neutropenia and the severity of oral or gastrointestinal mucositis in the early period following hematopoietic stem cell transplantation.
Disclosure of Interest: None declared.

P612
A supersaturated calcium phosphate solution – New hope for old problem. Single centre experience

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Introduction: Oral mucositis (OM) is a common complication of oncological treatment. It represents one of the most important dose-limiting toxicities of chemotherapy and occurs frequently after highdose chemotherapy with subsequent auto – and allogeneic haematopoietic stem cell transplantation (HSCT). It affects around 60-100% of patients undergoing conditioning regimens. Hitherto, there has been no uniform standard of care in prophylaxis and treatment of OM. Expert panel recommended basic oral care, systemic and local anaesthetics/analgesics, low-level laser therapy and cryotherapy for oral mucositis prevention and treatment, only one medication has gained a regulatory approval for the oral mucositis – a recombinant human protein that mimics endogenous growth factor for epithelial cells – palifermin. A new preparation has been recently registered, a supersaturated calcium phosphate solution – market name Caphosol®. It is used as a mouthwash in order to moisten and cleanse the oral cavity, including oral mucosa, tongue and the oropharynx. It is assumed that highly concentrated $Ca2+$ and

PO4^-3– ions diffuse to the epithelium interstitial matrix and help in maintaining mucosal integrity as well as help heal the deficits.
Materials (or patients) and methods: In this study the 3-year single institution experience with two OM treatment modalities in patients after high-dose chemotherapy with subsequent auto- and allogeneic (from a related donor) HSCT was reported. We especially asked whether Caphosol® is an effective mean to prevent and treat oral mucositis in HSCT patients. We analized 100 patient – 54 of them were treated with Calcium phosphate mouth rinse. The historic group (46pts) was composed of patient treated with HSCT before Calcium phosphate (Caphosol®) was available. The solution was administered 4 times daily, starting from the day before the beginning of chemotherapy till the end of hospitalization. The severity of oral mucositis was evaluated every day according to the WHO 5-grade scale. Number of days with painkillers (MF 4 times s.c.) and with total parenteral nutrition were compared. The patients' groups were comparable for statistical analysis in number of patients, their age, sex.
Results: Among patients treated with Caphosol and palifermin oral mucositis of III and IV degree was not observed. None of the patients needed total parenteral nutrition. Analgetics were used respectively: for Caphosol - in 9% of cases (duration time 0-7 days), for palifermin - in 10% of cases (duration time 0-3 days). In 40.9% of cases in the Caphosol group OM was not observed, in the group treated with palifermin 70% of cases were free of any kind of OM symptoms. In the control group OM was observed in all cases, 50% concerned grade 3 and 4. In comparison with the control group, using Caphosol and palifermin resulted in significant decrease: of the occurence of grade 3 and 4 OM, of the duration of OM and of the demand for narcotic analgesics [opioids].
Conclusion: Caphosol ® seems to decrease the incidence, severity and duration of OM. The differences were noticed in auto-HCST group, not in allo-HSCT group. It needs to be tested in randomized trials, because its easy administration and cost-effectiveness may render it a valuable addition to the standard care in the treatment of OM.
Disclosure of Interest: None declared.

P613
Interventions in allogeneic transplantation patients after day 100 till 1 year

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Introduction: Allogeneic transplantation is accessible to an increasing number of patients but is associated with significant complications. In the UK funding for transplantation only covers complications occurring within the first 100 days, but we increasingly see later issues arising in these patients. We report on a single centre experience of the interventions required in allograft patients from day 100 until 1 year.
Materials (or patients) and methods: We reviewed 76 consecutive allograft patients at 1 year post transplant for interventions occurring after 100 days. This included clinic attendances, DCU reviews and admissions and we analysed this against the frequency of acute and chronic GvHD, CMV reactivation, ECP, chemotherapy and drug delivery, blood product support, intrathecal chemotherapy, DLI infusions and infections.
Results: The mean number of hospital visits at the 6 month time-point was 14.4 (range 8-34), and at 1 year was 18.5 (range 5-64). At 6 months significantly more interventions were required in patients needing ECP for GVHD (mean 25 vs 14), Rituximab for ABO incompatibility (mean 21.8 vs 13.9), blood transfusions due to ABO incompatibility and relapse (mean 19.8 vs 13.8), Epo/GCSF for poor graft function (mean 17.9 vs 13.7) and drug delivery like foscarnet (mean 19.5 vs 13.5). At the 1 year time-point more interventions were required for those with relapse (mean 33.1 vs 15.8), requiring chemotherapy (mean 27.6 vs 15.8), cGvHD requiring ECP (mean 36 vs 16.7), poor graft function (mean 25.3 vs 15.6), bacterial infections (mean 22.6 vs 15.4), rituximab for cGvHD (mean 29.2 vs 17.7), drug delivery (eg foscarnet) (mean 27.6 vs 17.3) and those needing blood product support (mean 40.1 vs 16). 31/76 (40%) of patients required hospital admissions. 17 admissions occurred between 3 and 6 months and a further 14 by 1 year (range 1-3 times). Median hospital stay was 10 days (range 3-139 days) and totalled 620 bed days. 4 out of 76 patients required ITU admission and totalled 29 ITU bed days (median 7.2 days per patient).The median number of interventions was similar at 6 months and at 1 year in RIC, MA and Seattle conditioned transplants and irrespective of donor source (sibling, MUD, Cord and haploidentical). The mean number of interventions in MUDs was 15.1 at 6 months and 20.2 at 1 year (*P* value 0.014) indicating ongoing complications and the mean was 12.6 at 6 months and 16.3 at 1 year in the siblings. The cord transplants however had declining number of interventions from 6-12 m (mean 13.1 at 6 months vs 11.1 at 1 year, *P* value ns). The median age was 54 years and no effect of age nor the HCT-CI on the number of interventions was seen. Neither CMV reactivation, (which occurred in 8/39 at risk patients) nor ABO mismatch affected the number of interventions.
Conclusion: This study suggests that allograft patients are still a significant burden on healthcare resources after day 100 post-transplant with a median of 14.4 visits (clinic and day care unit reviews) at 6 months and 18.5 at 1 year and 620 hospital inpatient bed days. Interventions post transplant did not depend on patient age, HCT-CI, type of conditioning or stem cell source but more on the development of complications such as GVHD, poor graft function, infections and relapse.
Disclosure of Interest: None declared.

P614
Abdominal imaging performed for evaluation of early complications of stem cell transplant (SCT): A single centre survey

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Introduction: Abdominal X-rays (AXR), ultrasound scans (US) and computed tomographies (CT) are often performed for the evaluation of abdominal symptoms following stem cell transplant. We evaluated the indications and results of these investigations in stem cell transplant (SCT) patients in the first 100 days over a one year period
Materials (or patients) and methods: Retrospective evaluation of indications for AXR, CT and US imaging along with the results over a period of 100 days post-transplant in consecutive patients ($n = 121$) over 1 year (October 2013 to September 2014) in a single centre. There were 59/121 autologous SCT and 62/121 allogenic SCT. Inflammatory markers (C-Reactive Protein) and microbiology results in correlation with the imaging results were also recorded. Case notes were reviewed to see if these results influenced management
Results: 14/121 patients had AXR done, of which 4 went to have CT scan. AXR were reported as normal in 2/4 patients who had AXR prior to CT. All patients had abdominal pain, 5/14 had abdominal distension, 6/14 had diarrhoea and 3/14 had vomiting. Results showed that 7/14 AXR were normal, while 4 had features of dilated bowel loops and 3 had faecal loading. All patients with dilated bowel loops had CRP exceeding 100 and one of them had positive blood cultures.
US scans were performed in 12/121 patients. Indications for US were abnormal liver function tests in 6/12, which showed the presence of gallstones in 2 patients, 2 patients had splenomegaly, while one each had cholecystitis and fatty liver. 2/12 patients had renal impairment but US did not show obstructive uropathy. 2/12 patients had haematuria, and the US demonstrated echogenic debris in bladder in one patient

and hydronephrosis in the other. 1/12 had abdominal pain and distension, where the US showed faecal loading. The remaining 1/12 had pyrexia and abdominal pain; US showed splenomegaly and duodenal inflammation, went to have CT scan which showed normal duodenum.
14/121 patients had CT abdomen, of which 10/14 had allogenic SCT and 4/14 had autologous SCT. Indication for CT abdomen was abdominal pain in all patients, associated with diarrhoea in 3 patients. 8/14 had fever, 3/14 had abdominal distension, and one each had ascites, perianal ulcer and PR bleeding. 10/14 patients had abnormal CT results, of which 4 patients were demonstrated to have colitis, 2 noted with splenomegaly, one patient each with dilated gallbladder, and dilated common bile duct, and perianal abscess and thickened small bowels wall. Of the 4 patients with colitis, 1 patient had clostridium difficile infection. Perforation was excluded in all 4 patients
Conclusion: AXR, US, and CT scans are important tools for the assessment of early abdominal complications in post-transplant patients, usually for evaluation of abdominal pain and distension. It is important to use the right investigation to answer the clinical question in order to avoid wasting resources. AXR is helpful in patients with abdominal distension to detect dilated bowel loops and faecal loading while US scans is a good tool for the evaluation of renal and hepatobiliary system where clear results can influence the management. CT scans have helped to exclude perforation secondary to colitis in some patients but the incidence of severe colitis diagnosed in over 100 patients early post-transplant is quite low (4/14) in our case series
Disclosure of Interest: None declared.

P615
Analysis of factors affecting pre transplant pulmonary function tests and impact on overall Survival after allogeneic stem cell transplantation

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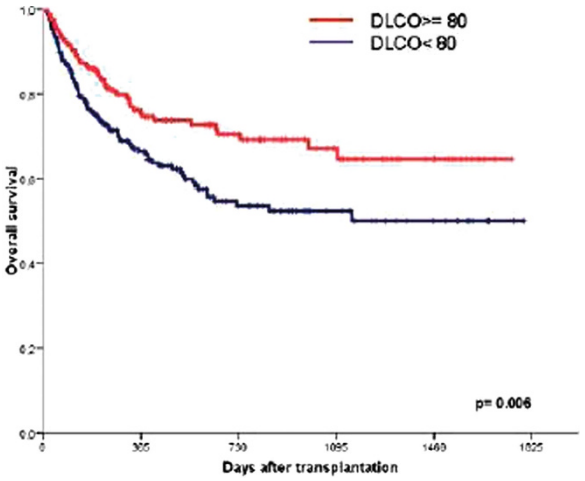
Introduction: The basal value of diffusing capacity of the lung for transferring carbon monoxide (DLCOc value, corrected by hemoglobin level) is a well recognized impact factor for non-relapse mortality in stem cell transplantation (SCT), and a relative contraindication for values $<50\%$. Around 40-50% of patients have a DLCOc $<80\%$ pre-transplant, and there is scarce literature about risk factors affecting this value.
Materials (or patients) and methods: We designed a retrospective and multicenter study with 365 patients undergoing to allogeneic SCT (alloSCT) in the last 5 years (median follow-up 23.7 months), to analyze the impact of pre-SCT variables on DLCOc, such as age, sex, basal disease, status at transplant, time from diagnosis to alloHSC, prior pulmonary infections, non-infectious complications and different previous treatments (for myeloid and lymphoid malignancies, respectively).
Results: Diagnosis was acute myeloid leukemias/myelodysplasia (AML/MDS) in 51.5%, followed by lymphoproliferative diseases (LPD) in 21.4%, others in 27%. 65% of patients had advanced disease status at transplant. DLCOc was $<80\%$ in 198 patients (54.2%). In univariate analysis significant factors for DLCO $<80\%$ were age (p:0.03), female sex ($P:<0.001$), prior radiotherapy (p:0.02) and prior non-infectious lung disease (p:0.06). In multivariate analysis the factors that remained statistically significant for decreasing DLCOc were female sex



($\beta = -8$; 95% CI, -11.6 to -4.4; $P<0.001$), smoking ($\beta = -5$; 95% CI, -8.9 to -1.1; $P = 0.01$), and a trend was found for prior SCT ($\beta = -4.8$; 95% CI, -9.8 to 0.2; $P = 0.06$) and previous non-infectious lung disease ($\beta = -5.3$; 95% CI, -11.5 to 0.8; $P = 0.09$). For LPD, significant factors decreasing DLCOc were female sex ($\beta = -10$; 95% CI, -18.3 to -2; $P = 0.01$), prior non-infectious lung disease ($\beta = -15$; 95% CI, -26.7 to -3.6; $P = 0.01$), R-CHOP ($\beta = -9.1$; 95% CI,-17.8 to -0.4; $P = 0.04$), and exposure to Gemzitabine (β: -8.2; 95% CI, -16.4 to -0.1; $P = 0.04$), whereas for AML/MDS, significant factors were female sex (β:-7.6; 95% CI, -12.6 to -2.6; $P = 0.003$), smoking (β: -7; 95% CI, -12.2 to -1.8; $P = 0.008$), and a trend for exposure to Ida-ARAC or FLAGIDA treatment ($P = 0.1$). Overall survival was significantly lower for patients with pre-SCT DLCOc $<80\%$ (66% at 1 year, 52.4% at 3 years) with respect to DLCOc $\geqslant 80\%$ (75.4% at 1 year, 67% at 3 years, p:0.006).
Conclusion: A low pre-transplant DLCOc has a significant impact on overall survival after alloSCT. Among the variables which significantly influenced on DLCOc, prior treatment should be closely monitored and even tailored for patients which are candidates to received an alloSCT.
Disclosure of Interest: None declared.

P616
Pretransplant corrected QT dispersion as a predictor of pericardial effusion associated with thrombotic microangiopathy after pediatric hematopoietic stem cell transplantation

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Introduction: Although pericardial effusion is one of the complications after HSCT; however, the underlying mechanisms and predicting factors have not been completely elucidated. Prolonged QT dispersion (QTD) and corrected QTD (QTcD) have been associated with serious arrhythmias and sudden death in many forms of heart disease. Recently

significant predictive utility of QTD and QTcD for heart failure and arrhythmia during and after preparative conditioning was reported in pediatric HSCT patients. However no study has evaluated the efficacy of QTD and QTcD to predict the development of pericardial effusion post-HSCT.
Materials (or patients) and methods: We did retrospective study about 89 children who underwent HSCT in single center to identify the risk factors for pericardial effusion, with particular focus on QTD and QTcD. The parameters of cardiovascular function were measured both before HSCT and at least once 60 days or more post-HSCT.
Results: Pericardial effusion occurred in 15 patients (cumulative onset rate: 17.4%) within 1 year post HSCT. The median onset delay post-HSCT was 61 days (range: 1–319). Among these 15 patients, 8 (9.2%) showed symptomatic pericardial effusion. Pericardial fluid exclusion method for urgent pericardiocentesis was performed in all 4 grade IV cases. Non-solid tumor disease, advanced disease, unrelated transplant donor, cord blood cell source, HLA mismatch, acute GVHD, chronic GVHD, and transplantation-associated thrombotic microangiopathy (TA-TMA) were significant risk factors for pericardial effusion in univariate analysis. Prior to HSCT, the following parameters did not significantly differ between pericardial effusion and no-effusion patient groups: total anthracycline dose, heart rate at rest, systolic function (LVFS), diastolic function (MV E/A ratio), and combined function (LV Tei index). In addition, there were no significant differences in QT and QTc prior to HSCT between the groups. However, pretransplant QTD and QTcD were significantly prolonged in the pericardial effusion group (QTD: 36.5 ± 15.7 ms vs. 25.9 ± 12.6 ms, $P = 0.006$; QTcD: 49.1 ± 16.7 ms vs. 32.4 ± 14.3 ms, $P < 0.001$). Multivariate analysis revealed that TA-TMA of $\geq$grade II (hazard ratio: 69.00, 95% CI: 10.50–451.00; $P < 0.001$) and prolonged QTcD (hazard ratio: 1.06, 95% CI: 1.00–1.13; $P = 0.049$) were significant independent risk factors for pericardial effusion. ROC curve of QTcD also predicted pericardial effusion after HSCT. A cut off value of 55.0 ms for prolonged QTcD had a sensitivity of 60.0% and a specificity of 93.7%. AUC was 0.777 (95% CI: 0.632–0.923).
Conclusion: The results suggest that patients with abnormally prolonged QTcD before the preparative regimen should be monitored by regular follow-up echocardiography to detect pericardial effusion, particularly when accompanied by additional risk factors for pericardial effusion.
Disclosure of Interest: None declared.

P617
Breast feeding after high dose chemotherapy and auto-SCT. Patients perspective, concerns and impact of above the diaphragm radiation therapy on milk production

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Introduction: Data are limited regarding prevalence of pregnancies after high dose chemotherapy (HDC) and auto-SCT. The data is even non-existing regarding breast feeding after auto-SCT. Because of high incidence of pregnancy and breast feeding post auto-SCT in our data (Akhtar et al, Bone Marrow Transplantation. 2013:48;2 S: S372-73. Abstract P1066), we explored the issue of breast feeding in our female patients.
Materials (or patients) and methods: Female patients who delivered after receiving HDC auto-SCT for non-Hodgkin (NHL) and Hodgkin lymphoma (HL) from 1997 to 2012 were identified. Data was also collected regarding breast feeding. We also asked 65 females about their perspective on safety of breast milk feeding for babies after receiving chemotherapy and HDC auto-SCT for lymphoma. A questinare was developed and administered by one interviewer either in person or on the phone with consent. This is a retrospective cohort analysis using our transplant data base (approved by the Institutional Review Board).
Results: We identified 26 females who became pregnant 50 times with 41 (82%) live births (2 still pregnant), 2/50 had birth defects. Median age at HDC was 24.8 years. Twenty-two/24 females who gave live birth breast fed their children. Six/22 used only breast milk as sole source for milk while 16/22 used breast milk + formula milk as supplement. Breast milk production was considered enough in 18/22 (82%). Five/22 (23%) used supplemental formula due to suboptimal milk production. Median duration of breast feeding was 4 months (range 1 to 24 months); 9 (41%) breast fed for <6 months, 4 (18%) 7-12 months and 2 (9%) 12-24 months (unknown for 7 women). Only 2 women claimed that they were stopped by her husband or family member from breast feeding, while 17/24 were encouraged to breast fed (no answer from 3 patients). Total of 15/22 patients who breast fed had received radiation therapy above the diaphragm and 5 of them had suboptimal breast milk production (4 to mediastinum and 1 to axilla). ''Enough'' breast milk production with diverse feeding frequencies were reported by 10/15 (66%) who had above the diaphragm radiation as compared to 7/7 (100%) who never received radiation therapy above the diaphragm ($P = 0.13$, Fisher exact test 2 sided). Issue of breast milk production and XRT cannot be further explored due to very small sample size. Out of eighty-nine females (<40 years) who had HDC auto-SCT, fifty-nine/89 (66%) considered breast milk as a safe option for the baby after being treated for lymphoma with chemotherapy and HDC auto-SCT while 17/89 (19%) did not (no answer from 10 patients).
Conclusion: Our report indicated that in Arab society, breast feeding, even after HDC auto-SCT is common and almost all patients practiced breast feeding. Most patients considered their milk production as enough for baby needs. Families supported and encouraged mothers for breast feeding. Breast milk was considered safe for the babies by most patients and families but some considered this unsafe. Suboptimal breast production in those who received above the diaphragm radiation warrants detailed analysis of large cohort to better understand this issue.
Disclosure of Interest: None declared.

P618
Characteristics and risk factors for hospital readmission in patients following hematopoetic cell transplantation

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Introduction: Patients who received a hematopoetic cell transplantation (HCT) (allogenic [allo-HCT]) or autologous [auto-HCT] are often readmitted after hospital discharge. However, these hospitalization episodes have been little studied.
Materials (or patients) and methods: We conducted a retrospective study to identify the risk factors for readmissions in recipients of HCT after 90 days of discharge, and their impact on the outcome. All consecutive patients receiving a HCT in our center between March 2009 and September 2014 were reviewed. Only patients discharged from the first admission were included. Readmission was defined as hospitalization for >24 hours within the first 90-days after discharge. Base line characteristics were compared using Chi-squared test. Univariate and multivariated analysis (MVA) of risk factors for readmission were performed using logistic regression.
Results: A total of 244 patients (92% of the 267 transplanted patients) were discharged during the study period (44% allo-HCT and 56% auto-HCT). Median time duration of the first

admission were 28 and 22 days for allo-HCT and auto-HCT respectively. Median age at HCT was 54 years (range 16-70) and median follow-up for survivors was 22 months (range 3-67). Allo-HCT indications included acute myeloid leukemia (34%) and acute lymphoblastic leukemia (22%) while for auto-HCT, patients were diagnosed with MM (40%) and DLBCL (17%). One hundred and four patients (42%) (27 [20%] auto-HCT; 77 [71%] allo-HC) were readmitted at a median of 12 days (range 1-89) after first discharge.The most common causes for readmission were fever ($n=$ 63, 61%) and gastrointestinal disorders ($n=$ 18, 17%). Median duration time of readmission was 7 days (range 1-127) (8 for allo-HCT and 3 for auto-HCT). Thirty-five patients (34%) had a microbiological documented infection. Among those receiving an allo-HCT, 15 (19%) patients were diagnosed with acute GVHD during readmission, and 14 (18%) had CMV infection. ICU admission was required in 14(13%). Eighteen patients (17%) (15 allo-HCT) died during second admission and 32 (38%) had a third hospitalization at a median of 18 days, mainly because of fever ($n=19$, 59%). In univariate analysis, risk factors for readmission were male gender, HTC-CI >2, days to neutrophil recovery, unrelated donor, base-line disease (acute leukemia) and type of transplant (allo-HCT). Risk factors for readmission in the MVA for the whole cohort were allo-HCT (vs auto-HCT) (HR 7.8 95%CI [4.3-14.1], $P<0.001$) and male gender (HR 2.2 95%CI [1.5-3.5], $P<0.001$). For the allo-HCT patients the only risk factor for readmission was male gender (HR 2.9 [95%CI 1.7-4.8], $P<0.001$). The duration of the first admission and the time to neutrophil recovery were not associated with a higher risk of 90-day readmission. Probability of overall survival at 2 years for the whole cohort and the allo-HCT patients were 67% (95%CI 64-70) and 63% (95%CI 58-68), respectively. Cumulative Incidence of NRM at 2 years for allo-HCT was 28% (95%CI 22-32). The only variable associated with lower NRM in the MVA was base-line disease (acute leukemia) (HR 2.3 [95%CI 1-9.6], $P=0.05$). Ninety-day readmission and the duration of the first admission were not associated with lower OS nor NRM.

Conclusion: Hospitalization within 90 days after HCT admission is frequent, especially after allo-HCT but it is not associated with worse outcome after the procedure.

Disclosure of Interest: None declared.

P619

High incidence of neurological complications after allogeneic stem cell transplantation: association with transplant related mortality

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Introduction: The incidence and outcome of neurological complications of patients undergoing allogeneic stem cell transplantation (alloSCT) remains to be established.

Materials (or patients) and methods: We have retrospectively studied all transplants ($n=89$) performed over a five year period (2009-2014) in our transplant center with the aim of characterizing all types of neurological events. Neurological complications were divided between early and late, whether they occurred before or after day 100, respectively.

Results: The overall incidence of neurological complications was 21% (19 episodes in 19 patients). Median age at alloSCT for patients developing neurological complications was 39 years (27-65) with 73% of patients ($n=13$) being male. Most frequent baseline diagnoses were acute leukemia (9) and plasma cell dyscrasia (4). At time of alloSCT, 11 patients (58%) were in CR, 3 (15%), 3 (16%) with progressive/refractory disease, 3 in PR and 2 (11%) untreated. No patient had developed CNS involvement by their disease prior to alloSCT, and 3 patients (15%) had a previous history of vascular CNS events (stroke, subarachnoid hemorrhage and intraparenchymal hemorrhage). Conditioning was myeloablative in 8 (42%) and reduced intensity in 11 (58%) patients. Over 95% of transplants were performed using peripheral blood stem cells from HLA-identical siblings ($n=10$, 53%) or matched unrelated donors ($n=9$, 47%). GvHD prophylaxis included Cyclosporine and Methotrexate in all cases plus in vivo T-cell depletion with Alemtuzumab ($n=8$) or ATG (n1 $=$) in all unrelated donor transplants.

The median interval between alloSCT and development of a neurological complication was 92 days (0-970). A total of 11 (57%) cases were classified as early. All but one case of peripheral neuropathy were CNS complications. We further classified the neurological complications as: 1) Infectious

[P619]

Neurogical Complication	Description	Timing	Status
Infectious	HHV-6 lymbic encephalitis	Early	Dead*
	VZV meningo-encephalitis	Early	Dead *
	Rhino-cerebral mucormycosis	Late	Alive
	Toxoplasma chorioretinitis	Late	Alive
	Staph. Aureus invasive rhinosinusitis	Late	Alive
Encephalopathic	Voriconazole-related	Early	Alive
	Metabolic	Early	Dead
	Metabolic	Late	Alive
	Metabolic	Late	Alive
Vascular	Pontine ischemia	Early	Dead *
	Intracranial hemorrhage	Early	Dead *
Epileptic	Tonico-Clonic seizure	Early	Alive
	Tonico-Clonic seizure	Early	Alive
Other	Reversible posterior leucoencephalopathy	Early	Dead*
	Cyclosporine-associated papilledema	Late	Alive
	Optic neuritis	Late	Alive
	Idiopathic leucoencephalopathy	Late	Dead
	Immune transverse myelitis	Late	Alive
	Drug-related polyneuropathy	Late	Alive

* Patients that died as a direct consequence of the neurological complication

($n = 5$; HHV-6 limbic encephalitis, VZV meningo-encephalitis, rhino-cerebral mucormycosis, toxoplasma chorioretinitis and Staph. Aureus invasive rhinosinusitis); 2) Encephalopathic ($n = 4$; one Voriconazole associated; three other metabolic causes); 3) Vascular ($n = 2$; pontine ischemia, intracranial hemorrhage); 4) Epileptic ($n = 2$) and 5) Other ($n = 6$, including one case each of reversible posterior leucoencephalopathy, Cyclosporine associated papilledema, optic neuritis, idiopathic leucoencephalopathy, immune transverse myelitis and drug-related polyneuropathy). See Table 1.
Five patients (26%) died as a direct consequence of their neurological complication. Two more patients died of additional causes resulting in an associated mortality of 37% (8% overall mortality for all transplants). The Kaplan-Mayer estimated overall survival at three years for patients that develop neurological complications was 62% with a median follow up of 37 months.
Conclusion: The incidence of neurological complications in our series was significant (21%), with a heterogeneous pathogenesis and was associated with a high mortality (37% amongst patients that developed any neurological event).
Disclosure of Interest: None declared.

P620
Incidence of secondary malignancies in recipients of allogeneic stem cell transplantation: a single centre experience

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Introduction: Recipients of haematopoietic stem cell (HSC) transplants have previously been reported to have an increased risk of secondary malignancy occurring in the post-transplant period. We retrospectively analysed data from a single centre cohort of 520 successively transplanted patients to establish local incidence figures.
Materials (or patients) and methods: The electronic records of 520 patients who received a HSC transplant at our institution were evaluated for transplant indication and protocol, stem cell source, age at transplant, duration of follow-up and occurrence of secondary malignancies. A total of 520 consecutive patients (51% male) received an allogeneic HSC transplant in Oxford between January 1st 1989 and October 1st 2014 and had evaluable electronic records with access to histopathology reports and follow-up documentation.
Results: The median age at transplant was 47 years (range, 5-74 years; 98% > 18 years), and the median follow-up was 1428 days (range, 17-8875 days). The majority of transplants was carried out for acute myeloid leukaemia (34.4%), followed by non-Hodgkin's lymphoma (14.8%) and acute lymphoblastic leukaemia (14.6%). Other indications included myelodysplastic syndrome (11%), chronic myeloid leukaemia (7.1%), myeloma (5.2%), chronic lymphocytic leukaemia (4.2%), other myeloproliferative conditions (4%), Hodgkin's Lymphoma (2.9%), and aplastic anaemia (1.7%). The stem cell source in the majority of cases was PBSC (77.2%), and in most cases (68.8%) a reduced intensity conditioning regimen was used. Just over half of patients (53.3%) had a sibling donor. At the time of follow-up, 53.3% of patients were still alive.
Forty patients (7.7%) were found to have 44 occurrences of a secondary malignancy; of these, 28 patients (5.4%) developed 32 cases of non-haematologic malignancy, mainly involving the skin. We documented thirteen cases of basal cell carcinoma, four cases of squamous cell carcinoma, and one case of melanoma. Other malignancies included two cases each of oesophageal carcinoma, adenocarcinoma of the bowel and carcinoma of the breast, as well as single cases of carcinoma of the cervix, endometrium, prostate, bladder, stomach, buccal mucosa, tonsil and lung. With regards to haematological malignancy we observed six cases of post-transplant lymphoproliferative disease, two cases each of myelodysplastic syndrome and Richter's transformation, and one case each of de novo myeloma and cutaneous T-cell lymphoma.
The estimated cumulative incidence rate of secondary malignancy overall was 10.4% (95% CI, 7.1-14.4%) at 10 years post-transplant. On univariate analysis, recipient age above 50 years, reduced intensity conditioning, PBSC as stem cell source, and transplant indication (acute leukaemia) were found to be significant predictors ($P < 0.05$) of developing a secondary malignancy, but on multivariate analysis none of these factors remained significant.
Conclusion: To date, there is only a small number of published reports on the occurrence of secondary malignancies in transplant patients. Our findings show a significant burden of secondary malignancy and demonstrate that long-term follow-up of transplant patients, with special attention to skin neoplasms, may be beneficial.
Disclosure of Interest: None declared.

P621
Elevate pretransplant serum ferritin levels are strongly correlated with poorer survival in patients with lymphoma that underwent autologous hematopoietic stem cell transplantation (autoHSCT)

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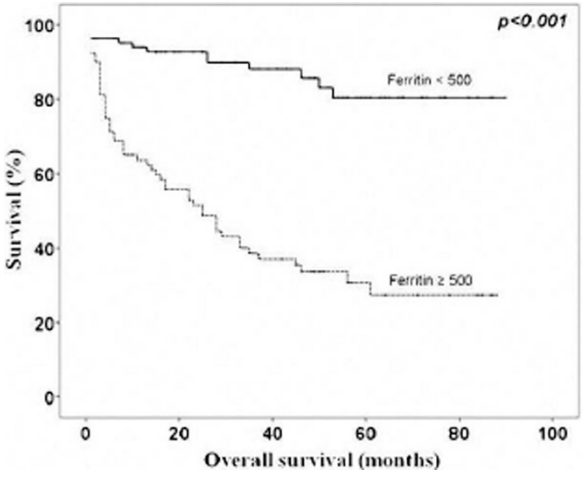
Introduction: Blood transfusions to correct anemia may cause undesired accumulation of iron, which is an important element for the function of human body and this iron overload also causes many complications ranging from mortality. Our goal was to evaluate the association between pretransplant ferritin levels and survival in patient that underwent autologous hematopoietic stem cell transplantation (autoHSCT).
Materials (or patients) and methods: 165 patients with lymphoma, who underwent autoHSCT between the years of 2007-2014, were included in study. Ferritin levels were used to determine iron status and the cut-off value was 500 ng/ml. The relationship between iron overload and survival was assessed by statistical analysis.
Results: In high-ferritin group, compared with low-ferritin group, median overall survival (OS) and disease-free survival (DFS) are both resulted inferior. (OS, 20 [range 1-88] vs. 42 [range 1-90] months; DFS, 10 [range 1-88] vs. 39 [range 1-90] months, respectively, $P = \ <0{,}001$). The number of patients



who survive was 101 (61.2%) and 73 of these were in low-ferritin group, 28 patients were in high-ferritin group. In 64 (38.8%) patients who died, 12 (14.1%) patients were in low-ferritin group, while 52 (%65.0) of them were in high-ferritin group ($P = <0{,}001$).
Conclusion: OS, DFS and 100-day mortality results were significantly lower in patients with high ferritin levels (≥ 500 ng/ml, $P < 0.05$).
Disclosure of Interest: None declared.

P622
Bone marrow iron stores (BMIS) might be used as a predictive marker for survival in patients with iron overload that underwent allogeneic hematopoietic stem cell transplantation (alloHSCT)

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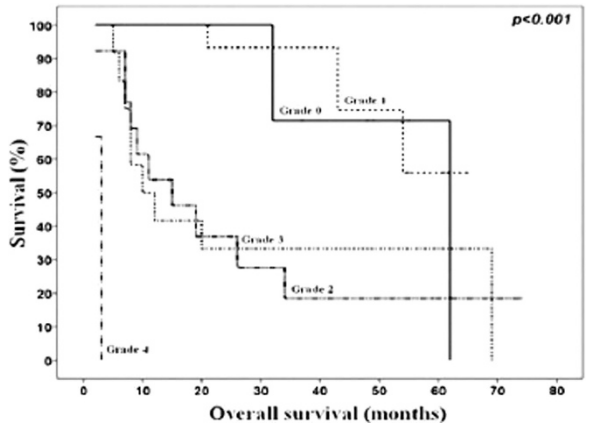
Introduction: Iron overload (IO) is one of the most signficant problems as a leading cause of death in patients with leukemia and those who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT).
Materials (or patients) and methods: We retrospectively evaluated the bone marrow iron stores (BMIS) in patients who underwent allogeneic hematopoietic stem cell transplantation ($n = 125$). The first available bone marrow biopsy specimens prior to the HSCT diagnosis or date of hospitalization (control group) were assessed in a blinded fashion using a standardized scoring system (1-4).
Results: A total of 125 patients were enrolled in the study. 76 (60.8%) of the patients were male and 49 (39.2%) were female. The median level of pre-transplant serum ferritin was 1023.00 ng/mL (min-max: 393.80-1627.50). 50 patients (40.0%) was died to primary disease or secondary complications (infection, bleeding) during post-transplant follow-up. The OS and DFS were strongly correlated with the degree of BMIS and both data were statistically significant ($P < 0.001$The majority of the patients were diagnosed with acute leukemia (83, 66.4%) and lymphomas (20, 16.0%). The median day for neutrophil engraftment was 14.00(min-max:13.00-16.00) days and 11.00 (min-max:10.00-14.00) days for platelet engraftment and $P = 0.012$, respectively)
Conclusion: The validation of BMIS for risk stratification in patients who undergo allogeneic hematopoietic stem cell transplantation may predict posttransplant outcomes.
Disclosure of Interest: None declared.



P623
Perceived quality of life in onco-hematological patients undergoing hematopoietic stem cells transplantation and association with medical, psychological and psychosocial variables

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Introduction: The most recent findings concerning perceived Quality of Life (QoL) in patients undergoing hematopoietic stem cell transplantation (HSCT) focus not only on the evaluation of QoL during the different phases of the treatment, but also on psychological, psychosocial and clinical variables potentially associated to the patients' perception of QoL. Particularly interesting is the evaluation of the association between the patient's ability to cope with stress determined by the illness (coping style) and his own perception of QoL during the different phases of HSCT, as far as specific intervention can be timely addressed to help patients to develop a more adaptive coping style. The aim of our study is to evaluate QoL before HSCT and also the correlation between QoL and the medical-clinical (diagnosis), psychological (coping modalities, stress level, anxious and depressive symptomatology) and psychosocial (age, gender, level of education) variables, measured during the pre-transplant phase.
Materials (or patients) and methods: From 2008 to 2011, 322 patients (41% female, 59% male; median age: 51, range 18-77; 54% acute leukemia, 23% lymphoma, 23% multiple myeloma) candidate to HSCT were studied. Self-administered questionnaires were used to evaluate QoL before HSCT patients [*Medical Outcomes Study SF-36* (Ware & Stewart, 1992)], anxious-depressive symptomatology [*Hospital Anxiety and Depression Scale HADS* (Zigmond & Snaith, 1983)], level of distress [*Psychological Distress Inventory PDI* (Morasso et al., 1996)] and coping styles [*Mental Adjustment to Cancer Scale MAC* (Watson et al., 1988)]. The association between QoL and the different variables already mentioned (see AIM) was studied in univariate analysis (statistical test SPSS-20).
Results: The analysis shows that patients perceive a significantly poorer QoL before HSCT, both mental and physical, compared to healthy population ($P < .05$, $P = .003$; $P = .001$). This perception varies depending on coping style adopted and on gender: adaptive coping styles and male gender are positively correlated with a better QoI ($P < .001$ for both). Lower perceived mental QoL is correlated with anxious-depressive symptomatology ($P < .001$). No other correlations were found between QoL and the analysed variables, including the diagnosis.
Conclusion: Confirming what already suggested in previous studies also in our series variables different from clinical ones are strongly linked with QoL.
These results show the need both to assess and to act on psychological variables in the pre HSCT phase in order to identify and help those patients at risk to experience poor QoL.
Specific interventions to develop adaptive coping strategies to face with the disease should be planned at the beginning of HSCT program in order to promote a better adjustment to the post HSCT phase, when patients' compliance and ability to manage with the disease is extremely important.
We are currently evaluating the relative role of various clinical variables and various psychological and psycho-social variables in defining perceived QoL and the efficacy of early interventions pre-HSCT directed to patients with maladaptive coping styles to ameliorate QoL.
Disclosure of Interest: None declared.

P624

Left ventricular systolic disfunction after T cell replete haploidentical transplantantion with post infusion Cyclophosphamide for haematological malignancies

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Introduction: Haploidentical T cell replete bone marrow transplant (haplo SCT) with post infusion cyclophosphamide (PT-Cy) is an emerging effective procedure for advanced haematological malignancies.
Materials (or patients) and methods: We retrospectively analyzed data focusing on cardiac toxicity in 79 consecutive haematologic patients after T cell replete haploidentical transplant and PT-Cy. Doppler echocardiography was performed for all patients before transplant, and after transplant only when clinically suggested. Clinical episodes were classified as early (before 100 days) and late (after 100 days). The aim of this study was to describe hearth toxicities after haplo SCT with PT-Cy
Results: From 2009, 79 patients were transplanted and 66 patients with an adequate follow-up were evaluable. The characteristic of the overall patient population are shown in Table 1.Overall, 15 patients (22%) with fatigue and tachycardia were diagnosed with left ventricular systolic dysfunction at Doppler echocardiography. The median time from transplant to cardiotoxicity was 120 days(range 15-774). 7 (10%) patients experienced early and 8 (12%) late. 3 out of 7 patients diagnosed in the early period are still alive without sequel and 1 patient died from disease relapse and 3 from toxicities other than cardiac. 2 out of 8 patients diagnosed in the late period experienced cardiologic symptoms after further treatment for relapse. 2 patients, in complete remission, died from hearth failure; 2 patients, retransplated after a previous allo, died from no-hearth related toxicity, and 2 patients are still alive in complete remission.
Conclusion: This retrospective analysis reported the onset of left ventricular systolic disfunction in 7 patients (10%) in the early phase and in 8 patients (12%) in the late phase after haplo SCT with PT-Cy. Cardiotoxicity after t replete haplo-transplant with post infusion CY has not been previously reported. As already known CY at a dose less than 1.5 mg/m2 did not correlate with cardiotoxicty. Due to the sample exiguity, it was not possible find a correlation with any clinical features. Probably the rate of left ventricular systolic disfunction is of a multifactors origin such as previous anthracicline exposure, mediastinal RT, ferritinemia value, pharmacological interactions between chemotherapy, immunosuppressive, antifungal drugs.
Disclosure of Interest: None declared.

Table 1. Patient characteristic

Sex	
M	10
F	5
Disease	
HD	6
NHL	8
AL	1
Conditioning	
RIC	14
MAC	1
Previous mediastinal RT	
Yes	3
No	12
Previous cardiotoxic treatment	
Yes	13
No	2
Pre transplant Ferritinemia	
>1000	6
<1000	5
Pre transplant FE	
>55%	10
<55%	5
Age	
<46	7
>46	8

P625

Cytomegalovirus associated hemorrhagic cystitis in patients following allogeneic HSCT: the clinical characteristics,antiviral treatment responses and risk factors

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Introduction: Late-onset hemorrhagic cystitis (LOHC) is a common complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT) and is primarily associated with viral infection. In our previous study, we found that cytomegalovirus is associated with LOHC following allo-HSCT. This study is to illustrate the characterization of patients with cytomegalovirus associated LOHC (CMV was prior to or at the onset of LOHC) and their response to antiviral treatment (Foscanet and/or Ganciclovir).
Materials (or patients) and methods: We conducted an institutional retrospective study to analyze the incidence and clinical factors associated with CMV-HC in allo-HSCT. With the diagnosis of LOHC, along with the general treatment, the empirical antiviral therapy with ganciclovir (5 mg kg/d) or foscarnet (80–120 mg kg/d) was given. For patients who did not respond to antiviral therapy, corticosteroids were adopted.
Results: During this period, we identified 38 (19%) patients with CMV-LOHC, with a median time of 34days (17-68 day) post-transplantation. The grade was as follows: grade 1($n = 13$, 34.2%); grade 2($n = 10$, 26.3%); grade 3($n = 14$, 36.8%) and grade 4 ($n = 1$, 2.6%). Following the general treatment along with the empirical antiviral therapy for nearly 2 weeks, 15 patients (39.4%) got complete response (CR), 9 patient (23.7%) received partial response (PR) and 14 patients (36.8%) got none response(NR). The overall response rate was 63.1% (CR + PR); In those patients with NR and PR state, 3 patients was got CR following the previous antiviral treatment. The other 20 patients was adopted with corticosteroids, and the response rate at 3days treatment was as follows: 3 patient got CR (5.6%), 12 patients got PR (66.7%) and 5 patients remain NR (27.7%). Both the univariate and the multivariate analysis showed that disease status pre-transplantation (HR = 8.477, 95% CI: 1.144-62.994, $P = 0.049$) and acute GVHD (HR = 35.823, 95%CI: 3.633-353.198, $P = 0.002$) were independent risk factors for the development of CMV-LOHC.
Conclusion: Cytomegalovirus-associated LOHC affects 19% of allogeneic HSCT patients. The empirical antiviral therapy with ganciclovir or foscarnet was important in the treatment of LOHC. As in the study, the independent risk factor for CMV-LOHC was disease status and the acute GVHD, the corticosteroids were used to control the immune response during the course.
Disclosure of Interest: None declared.

P626

Phase I study evaluating the pharmacokinetics and safety of Defibrotide for the treatment of veno occlusive disease in healthy male volunteers in Japan

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Introduction: Veno-occlusive disease (VOD) is serious complication of post stem cell transplantation (SCT) and associated with a high mortality rate (>80% in severe VOD). Defibrotide (DF) is a mixture of oligonucleotides that is effective in the treatment and prevention of VOD. DF is DF is approved for treatment of severe hepatic VOD in HSCT therapy by the EMA and is an investigational drug that has been granted Orphan Drug status by the FDA. This phase I study of DF to evaluate pharmacokinetics (PK) and safety was conducted in healthy subjects in Japan.

Materials (or patients) and methods: Ten healthy male volunteers who were enrolled in the phase I study received a single dose of 3 mg/kg, 6.25 mg/kg DF, or placebo, in consecutive dose escalation cohorts. Blood samples for PK analysis were obtained just before the start of the infusion; at 1 and 2 hours after the start of infusion; and at 5, 15, 30, and 60 minutes after the end of the infusion. The non-compartmental pharmacokinetic parameters were calculated for each subject, and population pharmacokinetic modeling was performed using NONMEM.

Results: No subjects experienced a dose-limiting toxicity. Mild hepatic impairment was observed only in the subjects who received 3 mg/kg DF. Serum concentrations of DF at 3 mg/kg were below the limit of detection of the assay. Mean pharmacokinetic parameters of DF by non-compartment model analysis were as follows: maximum concentration (Cmax; 20.59 +/- 4.11 mg/L), volume of distribution at steady state (Vdss; 7.73 +/- 1.25 L), elimination rate constant (ke; 1.55 +/- 0.33 /hr), total body clearance (CL; 9.269 +/- 1.175 L/hr), $AUC_{0\text{-}3}$ (37.09 +/- 7.82 mg · hr/L) and $AUC_{0\text{-}\infty}$ (42.32 +/- 6.95 mg · hr/L). Mean population pharmacokinetics parameters and the inter-individual coefficient of variation were as follows: CL; 0.145 L/hr/kg, Vd; 0.085 L/kg, ωCL; 15.4%, and ωVd; 20.5%.

Conclusion: DF was safe and well tolerated at both doses tested. This PK study of DF in healthy male volunteers demonstrated that PK properties of DF were similar to the reports of overseas literature. Pharmacokinetics of DF were not found to be associated with ethnic differences in Japanese healthy volunteers.

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P627

Incidence, risk factors, and outcome of early hemostatic complications in patients after allogeneic hematopoietic stem cell transplantation: a retrospective multicenter study of 551 patients

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Introduction: Hemostatic disorders are common and potentially fatal complications in patients undergoing allogeneic hematopoietic stem-cell transplantation. However, Limited data exists on early diagnosis and prevention of these complications.

Materials (or patients) and methods: In this study, 551 allogeneic transplantation recipients were enrolled to investigate the incidence, risk factors, and outcome of thrombotic or bleeding complications in the first 100 days after transplantation.

Results: Of all the patients, 261 cases (47.4%) developed bleeding events, the cumulative incidence of minor, moderate, and severe bleeding was 28.9%, 14.9% and 3.8%, respectively. The incidence of thrombotic complications was 4.5% (25/551 cases), consisting of 15 cases of veno-occlusive disease, 7 thrombotic microangiopathy, 2 pulmonary embolism, and 1 deep vein thrombosis. Risk factor analysis demonstrated that veno-occlusive disease, II-IV acute graft-versus-host disease and cord blood transplantation were independent predictors for bleeding complications in multivariant analysis and platelet counts less than 10×10^9/L were significantly associated with severe bleeding. Meanwhile, mismatched donor, polyomavirus BK infection, cytomegalovirus infection and II-IV acute graft-versus-host disease were potential risk factors for late-onset hemorrhagic cystitis, while total body irradiation conditioning regimen and high-risk disease status prior to transplantation were significantly associated with the occurrence of thrombotic disorders.

Conclusion: Severe hemorrhage and early-onset of thrombotic disorders independently increased the mortality of allogeneic transplantation recipients. However, late-onset hemorrhage cystitis did not appear to affect the survival rate significantly. Our study demonstrated that hemostatic complications following transplantation have much high mortality. Therefore, early diagnosis and therapeutic intervention of hemostatic complications are crucial to improve the outcome of allogeneic hematopoietic stem cell transplantation recipients.

Disclosure of Interest: None declared.

P628

A plasma marker panel predictive for transplant-related complications and survival

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Introduction: Non-infectious transplant-related complications (TRCs) following allogeneic hematopoietic cell transplantation (allo-HCT) are associated with unfavorable prognosis, but it's difficult to predict the survival outcome before transplant. Inflammatory endothelial injury and hemostatic abnormality were considered to play a critical role in the development of severe TRCs. This study aimed to explore plasma markers that could serve as independent predictors for dismal TRCs.

Materials (or patients) and methods: A total of 224 patients who received allo-HCT at two institutes between 2000 and 2013 were evaluated. TRCs within 100 days were categorized into three groups; complications with endothelial damage (ED), acute GVHD, and the other complications (OC). ED was defined as sinusoidal obstruction syndrome (SOS), transplant-associated microangiopathy (TAM), capillary leak syndrome

(CLS) and idiopathic pulmonary syndrome (IPS). Plasma angiopoietin-2 (ANG2), VEGF, soluble thrombomodulin (sTM), D-dimer, plasmin-α2 plasmin inhibitor complex (PIC), soluble IL2 receptor (sIL2R) and C-reactive protein (CRP) were measured. Multiple characteristics including plasma markers were evaluated for their association with the incidence of TRCs and the probability of overall survival (OS) and non-relapse mortality (NRM).

Results: Median follow-up duration of surviving patients after transplant was 3.9 years. The source of graft was bone marrow from unrelated donor in 40%, cord blood in 29% of the patients. Eighty-four percent of the patients received myeloablative conditioning regimen.

Accumulative incidence of total non-infectious TRCs was 70.4% at day 100. ED was observed in 62 patients: SOS ($n=19$), TAM ($n=28$), CLS ($n=10$) and IPS ($n=5$). Acute GVHD occurred in 108 patients: grade I ($n=33$), grade II ($n=44$), grade III/IV ($n=31$). OC developed in 32 patients.

When cumulative incidences of ED at day 100 were compared according to biomarker levels at transplant, the incidence was significantly higher in the high ANG2 ($\geq$1.8ng/ml; $P<0.001$), high sIL2R ($\geq$0.5U/ml; $P=0.0027$), and high sTM ($\geq$2.4ng/ml; $P=0.011$). When patients were stratified by the three markers, the incidence of ED was 63.8% (high ANG2 plus either high sIL2R or high TM; $n=47$), 10.8% (all low; $n=74$), and 27.9% (others; $n=68$). No significant difference was observed in the cumulative incidence of grade 3 to 4 acute GVHD at day100 between high- and low- groups of any biomarkers tested, although weak association was observed with ANG2 in a cohort of patients who developed acute GVHD.

With adjusted analysis, the 3-year NRM rate was significantly higher in high groups of ANG2 ($\geq$1.8ng/ml; HR 2.27; $P=0.0036$) and CRP ($\geq$1.9 mg/dl; HR 2.97; $P<0.001$). When patients were divided by a combination of the two markers, NRM was 12.4% (group A; both low; $n=115$), 28.9% (group B; either high; $n=82$), and 63.2% (group C; both high; $n=26$). The 5-year OS was also significantly different among the above three groups (A, 63.7%; B, 48.0%; C, 21.0%; $P<0.001$). Multivariate analysis revealed that group B (HR, 1.80; $P=0.0093$) and group C (HR, 2.93; $P<0.001$) along with disease risk and HLA-mismatch donor were independently associated with poor OS.

Conclusion: Combination of multiple biomarkers at transplant had significant powers for predicting the occurrence of ED and survival. The high predictability would make them useful for real-time clinical judgment and early intervention.

Disclosure of Interest: None declared.

P629

Second allogeneic haematopoietic stem cell transplantation using reduced-intensity conditioning regimen as treatment for haematological malignancies relapsing following first allogeneic haematopoietic stem cell transplantation

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Introduction: Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for haematological malignancies. But the management of patients relapsing after allo-HSCT is controversial. The use of a second allo-HSCT has been reported to be associated with improved disease-free survival compared with chemotherapy, cytokine therapy and donor leukocyte infusion. We performed a retrospective survey of second allo-HSCT for haematological malignancy relapse in our institution.

Materials (or patients) and methods: We have retrospectively analyzed the records of 333 adult patients who underwent allo-HSCT in our hospital between April 2005 and December 2014. Thirty-five patients were eligible for a second allo-HSCT, having relapsed after the first allo-HSCT.

Results: The underlying disease was acute myeloid leukemia (AML) in 22 patients, myelodysplastic syndrome (MDS) in 4, acute lymphoblastic leukemia (ALL) in 7, myeloproliferative disorder (MPD) in 1, and malignant lymphoma (ML) in 1. Ages ranged from 17 to 66 years (median 38); 10 patients were female and 25 were male. The median time to relapse following the first allo-HSCT was day +259 (range 42 - 2115). Twelve patients achieved a 2nd complete remission (CR), and 3 patients were in a 3rd CR at the second allo-HSCT. 20 patients were non-responders (NR) at the second allo-HSCT. All patients were treated with reduced-intensity conditioning regimens; fludarabine, melphalan and low-dose total body irradiation (TBI) containing regimens. The donor source was sibling bone marrow (BM) or peripheral blood stem cells (PBSC) in 8 patients (including HLA mismatched PBSC in 4), unrelated BM in 15, and unrelated cord blood in 12. Currently six out of 35 patients (17%) are alive and disease-free after second allo-HSCT (range 289 - 1837). All of these patients achieved a state of CR at the second allo-HSCT, had a remission lasting over 6 months following the first allo-HSCT and developed chronic graft versus host disease (GvHD). Transplant-related mortality (TRM) within day +100 occurred in 9 patients (26%). Twenty-one patients (60%) developed chronic GvHD. The median time to relapse following the second allo-HSCT was day +158 (range 69 - 525). In total, 29 patients died. The causes of death were relapse (17 patients; 59%), GvHD (3 patients), infection (6 patients) and other causes (3 patients).

Conclusion: Our study suggests that second allo-HSCT as treatment for relapse of haematological malignancy after first allo-HSCT is effective in selected patients who have achieved a CR state at second allo-HSCT and remission lasting for 6 months following the first allo-HSCT.

Disclosure of Interest: None declared.

P630

Late complications (LC) and quality of life (QOL) after allogeneic stem cell transplantation (allo-SCT)

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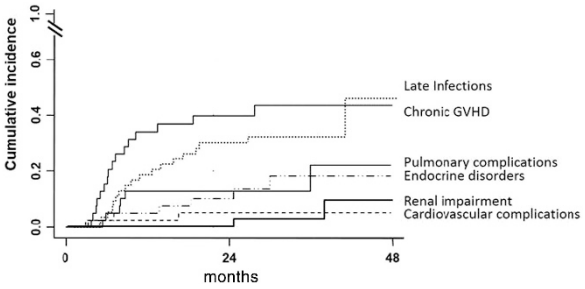
Introduction: While the features of early complications of allo-SCT are well known, data is still sparse in the setting of LC and especially long term QOL after allo-SCT.

Materials (or patients) and methods: This report analyzed the features of LC and QOL after day +100 in 39 consecutive patients who underwent allo-SCT in our institution and

[P629] Table. 1 Treatment and Clinical outcomes of long-term disease free survivors

Sex	Age	Diagnosis	Diagnosis at 2nd allo-HSCT	Donor source	conditioning regimen	GvHD prophylaxis	relapse following first allo-HSCT	chronic GvHD	following second allo-HSCT
female	54	AML	3rd CR	unrelated BM	Flu/Mel/TBI 2Gy	FK506 + short MTX	day +1055	Yes	day +1837
female	17	AML	2nd CR	unrelated BM	HDAC/Flu/Mel/TBI 2Gy	FK506 + short MTX	day +313	Yes	day +1769
female	27	ALL	2nd CR	unrelated cord blood	VP16/Flu/Mel/TBI 2Gy	FK506 + miniMTX	day +1070	Yes	day +1734
female	23	AML	3rd CR	unrelated cord blood	HDAC/Flu/Mel/TBI 2Gy	FK506 + miniMTX	day +738	Yes	day +1501
male	41	ALL	3rd CR	unrelated cord blood	VP16/Flu/Mel/TBI 2Gy	FK506 + miniMTX	day +1810	Yes	day +822
male	28	ALL	2nd CR	HLA miss-matched sibling PBSC	VP16/Flu/Mel/TBI 2Gy	FK506 + short MTX + ATG	day +652	Yes	day +289



survived for a minimum of 2 years after transplantation. QOL was assessed in a cross-sectional study by the use of EORTC QLQ-C30 and SF-36 questionnaires.

Results: The median age of 19 female and 20 male recipients was 44 (range, 18-58) years. In all, 12 patients (31%) had a lymphoid malignancy, while 27 patients (69%) were diagnosed with myeloid malignancies. In total, 27 patients (69%) received peripheral blood stem cells, while 12 patients (31%) received unmanipulated bone marrow. Twenty-five grafts (64%) were obtained from HLA identical siblings, 13 (33%) from HLA matched unrelated donors and 1 (3%) from a haploidentical donor. Twenty patients (51%) received a myeloablative conditioning and 19 patients received fludarabine, busulfan and ATG-based reduced-intensity conditioning regimen (49%). With a median follow-up of 945 days (range, 725-1451), chronic GVHD (cGVHD) was the most prevalent late complication with a cumulative incidence of 44% (95%CI, 27-59) at 2 years. Late infections, mostly viral, also had a cumulative incidence of 44% (95% CI 28-60). The cumulative incidence od organ-specific LC was 58% (95% CI 24-82%). Pulmonary complications were often related to cGVHD for a cumulative incidence of 22% (95%CI, 6-44). The cumulative incidence of cardiovascular complications was 5% (95%CI, 1-15) and of renal impairment 9% (95%CI, 1-28). Endocrine disorders had a cumulative incidence of 18% (95%CI, 7-34) involving thyroid dysfunction in 5% (95% CI 1-15). A secondary malignancy occured in one patient as metastatic pancreatic adenocarcinoma and led to the patient's death. In the univariate analysis, age of patients, type of conditioning, donor or source of the cells did not influence the incidence of LC. However, patients with grade II-IV acute GVHD had significantly ($P = 0.02$) higher cumulative incidence of organ-related LC (67%, 95%CI 28-88) compared to patients with grade 0-I acute GVHD (23%, 95% CI 8-43). In this series, 33 patients (85%) accepted to participate in the QOL survey. Among these, 15 patients (45%) had developed cGVHD after allo-SCT. Overall, patients had good global quality of life with a general health score of 50 (SD 18) in the SF-36 and mean global QOL group score of 62 (SD 22) in the QLQ-C30 questionnaire. Compared to the group without cGVHD, patients with cGVHD had significantly lower QOL in terms of emotional well-being and social functioning in the SF-36 questionnaire. Similarly, in the EORTC QLQ-C30, patients with cGVHD had significantly lower QOL in terms of emotional, cognitive and social functioning and reported significantly more financial disturbancies ($P < 0.05$ for all comparisons). Interestingly, there were no significant diferences in the terms of physical functioning and symptom scales between these two groups of patients.

Conclusion: In summary, patients who have clinically severe acute GVHD after allo-SCT have a higher probability of late organ-related complications. Among these complications, chronic GVHD remains to be a most prevalent problem that affects QOL, requiring long-term appropriate psychological support for patients.

Disclosure of Interest: None declared.

Conditioning regimen II

P631

Sequential chemotherapy associating Thiotepa, Etoposide and Cyclophosphamide followed by reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation for the treatment of refractory hematological malignancies

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Introduction: The results of conventional allo-SCT in refractory hematologic malignancies are poor. The sequential FLAMSA strategy has shown promising results in refractory AML. We developed a new sequential approach combining an induction chemotherapy associating Thiotepa, Etoposide and Cyclophosphamide (TEC), which has a broad anti-tumor activity, followed by RIC regimen for the treatment of wide spectrum refractory hematologic malignancies.

Materials (or patients) and methods: 22 patients with refractory hematologic malignancies received an allo-SCT after a TEC-RIC regimen. Patients received total dose Thiotepa 10 mg/Kg, Etoposide 400 mg/m^2, Cyclophosphamide 1600 mg/m^2 (D-15 to -10), and after a 3 days rest, Fludarabine 150 mg/m^2, iv Busulfan 6.4 mg/Kg (D-6 to -2) and Thymoglobuline 5 mg/Kg (D-3 and -2). Graft versus host disease (GVHD) prophylaxis consisted in Cyclosporine and Mycophenolate Mofetil. High dose Cyclophosphamide post-transplant (PT-CY) was added in case of a haplo donor. Prophylactic DLI was scheduled by D120 after withdrawal of immunosuppression.

Results: Median age was 44 years (range 17-65). All patients had refractory hematologic diseases: 11 AML, 6 ALL, 3 CMML and 2 DLBCL. 80% leukemic patients had persistent marrow blasts at transplant (median $= 20\%$) and 10/11 AML had poor cytogenetic. Sequential allo-SCT was performed after a median of 2 lines of prior treatment (range 1-4) including 2 auto and 3 allo-SCT. Five patients were transplanted with a sibling donor, 9 with an unrelated HLA 10/10 ($n = 7$) or 9/10 ($n = 2$) donor and 8 with a haplo-identical donor. Graft source was peripheral blood stem cells in 86%. Median follow up was 6.5 months (range, 1.5-20). All patients engrafted, median time for neutrophils and platelets recovery were 14 (range, 11-25) and 11 (range, 7-50) days, respectively. Toxicities of conditioning were all reversible and included 54% of mucositis (median grade $= 2$), 1 case of VOD, 4 other grade 1-2 liver toxicities (18%) and 5 grade 1 to 3 renal toxicities (22%). CMV and EBV reactivations occurred in 54% of patients, all of them responded to preemptive treatments, and 40% of patients developed hemorrhagic cystitis with positive BK virus, independently of the use of PT-CY. At D30, median blood chimerism was 99.5% donor (range, 96-100) and all patients reached complete morphological remission. Cumulative incidence of grade II-IV and III-IV acute GVHD at day 100 were 32% and 22%, respectively. Among 15 patients evaluable after D100, 7 developed chronic GVHD. Non relapse mortality (NRM) was 11% at D100 and 17% at 6 months. Relapse incidence was 30% at 6 months and 1 year. Eight of 13 patients evaluable after D120 without relapse (61%) received preemptive DLI at a median of 153 (range, 90-216) days, 6 of them were alive in CR at last follow up. Eight patients died, 3 of relapse, 2 of infection and 3 of GVHD. At 1 year, the probability of overall survival was 45%. There was no difference in outcomes between haplo and non haplo-SCT.

Conclusion: We describe a relatively safe and highly effective sequential conditioning regimen prior to allo-SCT that could be proposed to any type of refractory and high risk

malignancy. A prospective study based on this new sequential approach including post-transplant immuno-intervention is currently being planned.
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P632
Cyclophoshamide following targeted oral busulfan (CY/BU) as conditioning for allogeneic hematopoietic stem cell transplantation in 20 patients with acute myeloid leukemia

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Introduction: Busulfan followed by cyclophosphamide (BU/CY) is commonly used high-dose conditioning regimen in allogeneic hematopoietic cell transplantation (SCT). However, liver toxicity and hepatic veno-occlusive disease (VOD) are frequent life-threating complications. According to some authors (*Cantoni et al., BMT, 2011: 344-349; Rezvani et al., BBMT, 2013: 1033-1039)* less liver toxicity and better outcomes were observed with the reversed order of CY and BU. Here we present our experience with combination CY/BU as conditioning regimen in cohort of 20 patients (pts) with acute myeloid leukemia (AML) before allogeneic SCT.
Materials (or patients) and methods: We analyzed 20 pts with AML undergoing myeloablative regimen CY/BU, consisted of intravenous cyclophosphamide 60 mg/kg/day on days -7 and -6 and oral busulfan (4 mg/kg/day) for four consecutive days (-5 and -2). Monitoring of busulfan levels was performed with steady state plasma concentration of 800-900 ng/ml in all pts. The GvHD prophylaxis consisted of cyklosporin A and methotrexate, pts with unrelated donor received antithymocyte globulin (ATG Fresenius) at 20 mg/kg/day on days -3 to -1. Median age was 31 years (range 18-47). Types of donors and used grafts were as follows: HLA identical sibling, $n = 5$; unrelated donor, $n = 15$; PBSCs, $n = 20$.
Results: Complete remission was achieved in 18 of 20 pts (90%), progression was presented in 2 pts (10%). Complete chimerism was achieved in 65% of pts (13/20). The median time of neutrophil engraftment (above 0.5x10E9/L) was 15 days, all pts engrafted. Nonrelapse mortality (NRM) after 100 days, 1 year and 2 years were 0%, 10% and 15%, respectively. Causes of death were refractory GVHD ($n = 1$) and infections ($n = 2$). The most frequent toxicities were grade III/IV infections according to common toxicity criteria in all 20 pts and gastrointestinal toxicities (grade III in 11 of 20 pts). No case of VOD was observed. Incidence of acute GVHD was evaluated in 20 pts: 55% (11/20) of pts had GVHD (grade I + II in 7 pts, grade III in 4 pts). Incidence of chronic GVHD was evaluated in 20 pts, 50% (10/20) of pts had GVHD (limited in 7 pts, extensive in 3 pts). With median follow-up from SCT 25 months (range 3-48), 70% of all pts (14/20) were alive (13 pts in remission, 1 pt with relapse), 6 pts died (3 deaths from NRM, 3 deaths from progression of AML). Five relapses (28%; 5/18) occurred after SCT.
Conclusion: Conditioning CY/BU with allogeneic SCT seems to be promising approach to the treatment of AML. It provides a combination of effective disease control and acceptable toxicity, no case of VOD occured. Event-free survival and overall survival at 2 years from SCT in our cohort of pts were 62% and 70%, respectively.
Disclosure of Interest: None declared.

P633
Do different antithymocyte globulin brands differently affect immune reconstitution after hematopoietic stem cell transplantation?

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Introduction: Few data are available on the differences between antithymocyte globulin (ATG)-Genzyme (ATG-G) and ATG-Fresenius (ATG-F) treatment on immune reconstitution after hematopoietic stem cell transplantation (HSCT). We report our data on immune recovery after ATG-conditioned HSCT in adult patients, comparing ATG-brand-dependent effects at currently applied dosages.
Materials (or patients) and methods: CD3 + T cell, CD4 + T cell, CD8 + T cell, NK cell and B cell counts were determined by flow cytometry on day 30, 60, 100, 180, 270, and 360 after myeloablative HSCT in 119 patients transplanted from 2006 to 2013 for acute myeloid ($n = 89$) and lymphoid ($n = 30$) leukemia. Bone marrow ($n = 34$) or peripheral blood ($n = 85$) stem cells were obtained from HLA-matched related ($n = 38$) or unrelated ($n = 81$) donors. Eighty-one patients (68%) received ATG as part of the conditioning: ATG-F 10-30 mg/kg was used in 45 (38%) of them, whereas 36 (30%) received ATG-G 4.5-7.5 mg/kg.
Results: We first compared lymphocyte subsets after ATG-based and non-ATG-based conditioning. CD3 + T cell recovery was influenced by ATG treatment only at the earliest time points (D30: 257 ± 35 cells/μl ATG, 351 ± 53 cells/μl no-ATG, $P = 0.017$; D60: 383 ± 57 cells/μl ATG, 720 ± 168 cells/μl no-ATG, $P = 0.033$). Within CD3 + T cells, ATG specifically affected CD4 + T cell recovery, with significantly lower counts throughout all the observation period. Although complete B cell reconstitution was observed by D180 in both groups, no-ATG patients reached higher counts at 1 year (201 ± 30 cells/μl ATG, 349 ± 71 cells/μl no-ATG, $P = 0.033$). Recovery of CD8 + T cells and NK cells was similar between ATG and no-ATG. Next, we performed a 3-group analysis by dividing ATG patients according to ATG brand. ATG-G group exhibited significantly lower CD3 + T cells at 1 month (185 ± 43 cells/μl ATG-G, 284 ± 37 cells/μl ATG-F, 351 ± 53 cells/μl no-ATG, $P = 0.0026$; ATG-G vs. ATG-F: $P < 0.05$), a difference not evident at subsequent time points. Short and long term recovery of the CD4 + subset confirmed to be strongly impaired after ATG, but without difference according to ATG brand. Early recovery occurred for NK cells in each group, but NK counts were significantly higher in ATG-G patients at 1 and 2 months post-HSCT (D30: 250 ± 33 cells/μl ATG-G, 156 ± 20 cells/μl ATG-F, $P < 0.05$; D60: 262 ± 39 cells/μl ATG-G, 149 ± 30 cells/μl ATG-F, $P < 0.05$), although similar to no-ATG patients (D30: 183 ± 33 cells/μl; D60: 207 ± 28 cells/μl). Multiple comparison analysis did not reveal any difference for CD8 + T cell and B cell reconstitution. No difference in the proportion of EBV reactivation was found among evaluable patients in ATG-G (10/39, 26%), ATG-F (8/45, 18%), and no-ATG (3/28, 11%) groups. Only 1 patient, treated with ATG-F, developed PTLD.
Conclusion: Current applied dosages of ATG-G and ATG-F similarly impair CD4 + T cell reconstitution after myeloablative HSCT, with no significant long-term effect on other immune cell subsets. We found no different impact of ATG use and brand on EBV reactivation and PTLD. Sparing of NK cells at early time points is specific for ATG-G, as ATG-F has a different spectrum of targeted antigens. A stimulatory effect of the ATG-G polyclonal serum on NK-lymphopoiesis expansion, rather than a compensatory reaction to T-lymphopenia, may explain higher NK cell counts. This finding may be of clinical relevance, given the role of NK cells in the graft-versus-tumour effect.
Disclosure of Interest: None declared.

P634

Increased risk for relapse if ATG are included in reduced intensity conditioning HSCT with sibling donors

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Introduction: The impact of *in vivo* T-cell depletion on transplantation outcomes in patients transplanted with reduced-intensity conditioning (RIC) remains controversial.
Materials (or patients) and methods: Initially we used ATG (Thymoglobulin) 8 mg/kg ($n = 21$) as part of the Fludarabin + busulphan RIC protocol before sibling donor HSCT. ATG was then removed as high incidence of relapse was seen ($n = 21$). All patients received CsA and MTX as GVHD prophylaxis. Most patients had AML or MDS with a median age of 58 years (32-72).
Results: Overall survival at 3 years was 43% in patients receiving ATG and 83% in patients not given ATG ($P = 0.015$). Non-relapse mortality (NRM) was 10% and 6% at one year in the two groups, respectively. No NRM before day 100 occurred. The cumulative incidence of relapse at 3 years was 52% in ATG treated patients and 11% in patients not receiving ATG ($P < 0.05$). Relapse-free survival (RFS) at 3 years was 38% and 83% ($P = 0.01$) in the two groups, respectively. Cumulative incidences of acute GVHD grades II-IV was 19% and 43% ($P = 0.13$), grades III-IV 6% and 16% ($P = 0.25$) in the ATG and non-ATG groups, respectively. Chronic GVHD was 48% and 82% in the two groups ($P = 0.14$), respectively.
Significantly more patients had reached full donor chimerism within the CD3 + and CD19 + cell lineages at three month after HSCT in patients without ATG treatment.
Conclusion: This small study suggests that high dose ATG (8 mg/kg) in sibling donor RIC HSCT increase the risk for relapse and reduce overall survival. Total removal of ATG produced excellent results.
Disclosure of Interest: None declared.

P635

Comparison of three TBI-based myeloablative conditioning regimens for allogeneic stem cell transplantation of adult patients with acute lymphoblastic leukemia (ALL)

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Introduction: Allogeneic stem cell transplantation (SCT) is a curative option for adult patients with high-risk acute lymphoblastic leukemia (ALL). However, the optimal condition regimen has not yet been defined. We performed a retrospective single center analysis for three myeloablative regimens based on 12 Gy total body irradiation (TBI) combined with either (1) high-dose Etoposide (E 60 mg/kg), (2) Cyclophosphamide (Cy 2x60mg/kg), or (3) moderate E (2x15 mg/kg) combined with Cy (2x60 mg/kg) according to Shigematsu et al. (2011).
Materials (or patients) and methods: From 1/2004 until 5/2014 sixty-five consecutive patients with B- ($n = 48$) and T-lineage ($n = 17$) ALL were transplanted without or beyond 1. CR ($n = 22$) or in 1. CR of high-risk disease ($n = 43$) including BCR-ABL + ALL ($n = 19$). All patients have been risk-stratified and treated according to the GMALL 07/2003 trial. 7, 45, and 13 patients were conditioned in groups E, Cy and E + Cy, respectively. Gender (female 29%, 33%, and 54%), patient age (median 32, 30, and 37 years), stem cell source (PBSC 100%, 91%, 100%), CD34 + cell number/kg (median $5.4x10^6$, $7.1x10^6$, and $5.8x10^6$), post-transplant GvHD-prophylaxis and stage of disease were balanced among the groups. Donors were different since only HLA-identical siblings were included into group E but were balanced in the Cy and E + Cy groups. ATG was given in 43%, 91% and 85%, respectively. The median follow-up was 0.8 (0.1-10), 3.7 (0.1-10.6), and 1.0 (0.3-1.8) years. We analyzed overall survival (OS), disease-free survival (DFS), treatment related mortality (TRM) and acute and chronic GvHD incidence using Kaplan-Meier statistics.
Results: Median survival of surviving patients (OS) was 42.9%, 73.3% and 84.6% at 12 months ($P = 0.12$) and 28.6%, 68.9% and 56.4% at 18 months ($P = 0.058$) for the E, Cy and E + Cy groups. DFS was 28.6%, 60%, and 56,2% at 12 months ($P = 0.35$) and 28.6%, 53,3% and 56.2% at 18 months ($P = 0.39$). TRM was 57.1%, 15.9% and 15.4% at 12 months ($P = 0.024$) and 57.1%, 21.0% and 15.4% at 18 months ($P = 0.047$). Acute GvHD III-IV° occurred in 57.1%, 13.3% and 38.5% patients ($P = 0.004$) and extensive chronic GvHD in 28.6%, 15.5% and 0% ($P = 0.13$) in the E, Cy and E + Cy groups. Acute GvHD ($n = 1$) and/or infectious complications ($n = 4$) resulted in high TRM in the E group (4/7).
Conclusion: Our data revealed an unexpected high TRM in the E group, and therefore conditioning with 12 Gy TBI and high-dose Etoposide has been stopped in our institution. In contrast, the combined radio-chemotherapy with a moderate Etoposide dose together with Cy is well tolerated and there is no additional toxicity as compared to the Cy group so far. With the limitation of short follow-up TBI/Etoposide/Cyclophosphamide conditioning in combination with ATG seems promising for allogeneic SCT in adult high-risk ALL patients and warrants further clinical evaluation.
Disclosure of Interest: None declared.

P636

Prospective evaluation of FLAMSA sequential chemotherapy followed by reduced intensity conditioning and allogeneic hematopoietic transplantation for high risk acute myeloid leukemia patients

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Introduction: Advances in chemotherapy have improved the prognosis of AML patients, however, high-risk patients still have a poor outcome and the only therapeutic strategy with curative potential remains allogeneic hematopoietic stem cell transplantation.
We conducted this prospective study in high-risk AML patients with the aim to improve the effect of allo-HSCT by sequential use of chemotherapy followed by reduced intensity conditioning (RIC).
Materials (or patients) and methods: The high-risk population included intermediate II and unfavourable patients (Dohner et al. Blood 2010), secondary AML, and patients requiring 2 induction courses to obtain CR. The FLAMSA sequential regimen consisted in fludarabine 30 mg/m^2, high-dose cytarabine 2 g/m^2, and amsacrine 100 mg/m^2 from days -12 to -9. After 3 days of rest, RIC consisted of 4 Gy total-body irradiation (TBI) on day -5, cyclophosphamide (40 mg/kg with HLA-identical sibling, 60 mg/kg for unrelated or mismatched donors) on days -4 and -3, and rabbit ATG (5 mg/kg total dose) from day -3 to day -1. In a group of patients, TBI was replaced by iv. busulfan (BU) 3.2 mg/kg/d during either 4 or 2 days according to patient age (> 55 years) (from day -7 to -4 or from day -5 to -4). GvHD prophylaxis consisted in cyclosporine from day − 1, and MMF (15 mg/kg bid) from day 0. Except for CB transplantation, patients received 3 prophylactic increased doses of donor lymphocyte infusions (DLI) if they were in CR and GvHD-free at day + 120 or 30 days after discontinuation of immunosuppressive agents starting at $1x10^6$ CD3 + cells/kg. Between January 2007 and December 2013, 66 patients were included; 33 males and 33 females with a median age at allo-HSCT of 52 years (range: 19-66), 59 (89%) were *de novo* and 7 (11%) secondary AML. At transplantation, 22 (33%)

patients were in CR (20 CR1 and 2 CR2) and 44 (67%) were in less than CR. Stem cell source was PBSC for 52 (79%) patients, CB for 6 (9%) and BM for 2 patients. Donors were 10/10 HLA matched siblings in 24 (36%) patients, 10/10 HLA matched unrelated in 18 (27%) patients and HLA mismatched for the rest of patients [unrelated 9/10 ($n = 18$), CB 4/6 ($n = 6$)]. For ABO compatibility, 32 (48%) were compatible, 13 (20%) had minor incompatibility and 21 (32%) had major incompatibility. For conditioning, 49 (74%) patients received TBI, 17 (26%) received BU.

Results: After transplantation, 59 (89%) patients engrafted, 7 patients did not engraft and died early (2 from relapse and 5 from infection). At day 90 post-allo-HSCT, 37 (71%) among 52 evaluated patients showed total donor chimerism. There were 24 patients with acute GvHD [10 gr I, 3 gr II and 7 gr III and 4 gr IV] with a cumulative incidence (CI) of 27% for grade $\geq$II; and 17 chronic GvHD [10 limited and 7 extensive], among them 5 after DLI, with a CI of 48% at 2 years. The median follow-up was 7 months (range: 0.1-76). Patients in CR had significantly better OS and CI of relapse at 2 years compared to patients in less than CR with 45% versus 18% ($P = 0.013$) and 27% versus 78% ($P = 0.001$) respectively. No statistical difference was found in outcomes of TBI compared to BU conditioning.

Conclusion: Patients in CR showed very promising results and could benefit the most from this strategy. A high rate of deadly infections was observed, thus an efficient prophylactic anti-infectious strategy is recommended.

Disclosure of Interest: None declared.

P637

Allogeneic SCT after Treosulfan, Etoposide, and Cyclophosphamide for Patients with ALL not eligible for TBI-Containing Regimens: A Phase II-Study of the German ALL Study Group (GMALL) and the German Cooperative Transplant Study Group

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Introduction: Total-body-irradiation (TBI) based preparative regimens are considered as standard conditioning therapy for allogeneic stem cell transplantation (AHSC) in patients with acute lymphoblastic leukemia (ALL). Within a multi-center prospective phase II study we have investigated the toxicity and efficacy of a non-TBI-based regimen consisting of treosulfan, etoposide, and cyclophosphamide in patients with ALL.

Materials (or patients) and methods: Inclusion criteria were complete remission, non-eligibility for TBI or patient's wish to avoid TBI. Between July 2007 and August 2010, 50 patients with a median age of 46.5 years were enrolled at ten German centers. 74% of the patients were in 1. CR and 26% 2. or higher CR.The conditioning regimen consisted of treosulfan (12 g/m^2) given intravenously on three consecutive days (-7, -6, and -5) plus etoposide (30 mg/kg BW) infused on day -4, and cyclophosphamide (60 mg/kg BW) intravenously on day -3 and -2. GvHD prophylaxis consisted of ATG-Fresenius (Fresenius Biotech, Gräfelfing, Germany), 20 mg/kg on day -3, -2, and -1 for unrelated donors, and optional for matched related donors. All patients received cyclosporine A and short course methotrexate (days 1, 3 and 6). Donors were HLA-identical sibling ($n = 8$), matched ($n = 42$) or mismatched ($n = 10$) unrelated.

Results: Primary graft-failure was observed in three patients. The toxicity was moderate including VOD in four patients. Acute graft-versus-host disease (GvHD) grade II - IV and grade III/IV was noted in 53% and 14%, respectively. Chronic GvHD at one year was seen in 41%, which was extensive in 14%. After a median follow-up of 24 months, the cumulative incidence of non-relapse mortality (NRM) at one year was 8%, and of relapse 36% and 51% at one and two years, respectively. Patients in first complete remission showed a 12-months relapse-rate of 23% compared to 69% in patients beyond first complete remission. After 24 months, the respective rates were 34% compared to 92%. The estimated 2-year disease-free and overall survival was 36% and 48%, respectively. Patients in first complete remission experienced a median DFS of 25.7 months versus 8.9 months in patients beyond first complete remission. The 12- and 24-months DFS-rates were 69% and 50%, respectively, compared to 23% and 0%, respectively.

Conclusion: Overall, we conclude that a conditioning regimen containing treosulfan, etoposide, and cyclophosphamide resulted in a low NRM, but a high risk of relapse in 2. or higher complete remission. This regimen might represents an alternative therapy for patients with ALL in 1.complete remission who need allogeneic stem cell transplantation but are not eligible for total-body irradiation. (registered under NCT00682305)

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P638

Retrospective comparison of two myeloabalative conditioning regimens in patients with AML/MDS: a monocentric experience

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Introduction: Myeloablative reduced-toxicity conditioning (MAC-RTC) have been developed to reduce the toxicity of standard myeloablative conditioning combining busulfan and cyclophosphamide (BuCy) without compromising the control of minimal residual disease, thus allowing allogeneic hematopoietic stem cell transplantation (HSCT) in elderly or those with comorbidities. For this purpose, cyclophosphamide was switched with fludarabine (FB).

Materials (or patients) and methods: We retrospectively analyzed post-transplantation results of patients receiving either standard myeloablative conditioning (Bucy), or a myeloablative reduced-toxicity conditioning (FB).

Results: 49 patients receiving HSCT for AML (39) or MDS (10), from February 2008 to October 2013, were included in this study. 19 were conditioned by BuCy, 30 by FB. 15.8% of BuCy patients were 50 years or older, 40% in the FB group ($P = 0.073$).

The 1 year overall survival and relapse-free survival were not significantly different between the 2 groups (OS 63.2% vs 66.7%, $P = 0.428$; RFS 63.2% vs 63.3%, $P = 0.99$). The 1 year transplant-related mortality (TRM) was 21.1% in BuCy patients, and 10% in FB. There was no significant difference between the 2 arms for the duration of aplasia (BuCy and FB: 16 days; $P = 0.690$). The median time of platelet recovery was significantly higher in the BuCy arm (BuCy: 11 d vs FB: 9 d; $P = 0.015$). Total chimerism was achieved in 84,2% in BuCy arm and 90% in FB arm ($P = 0.665$). Complete donor chimerism was obtained faster in FB arm (31 days vs 59).

The most common toxicity was mucositis (grade $\geq$ 3 in 30% of patients). 13.3% of patients receiving FB experienced liver toxicity $\geq$ grade 3, and 6.7% veno-occlusive disease (VOD) (2 patients, including 1 death). In the BuCy group, no patient had liver toxicity. There was no significant difference between the cumulative incidence of kidney, liver, and gastrointestinal toxicities between the 2 groups ($P = 0.363$, 0.148 and 0.688, respectively). The percentage of reactivation of CMV was not significantly different (BuCy: 31.6% vs FB: 30%; $P = 0.907$). EBV reactivations tended to be more frequent in the FB arm (46.7% of patients vs BuCy: 21.1%; $P = 0.07$). aGVH grade II-IV was observed in 52.6% of BuCy patients, 46.7% in FB ($P = 0.684$). In BuCy group, 27.8% of patients presented extensive cGVH vs 39.6% in the FB group ($P = 0.639$).

Conclusion: This study shows that the FB conditoning allows myeloablation in elderly patients, thus retaining a good control on residual disease, without increased toxicity when compared to classical MAC. However, our study doesn't reach statistical significance for some criteria (as TRM, RRT, GVHD) regarding the small number of patients enrolled. Larger prospective studies are precluded to answer the question of toxicity criteria.

Disclosure of Interest: None declared.

P639

Generic (Bioequivalent) Melphalan Has Similar Efficacy and Toxicity but Lower Cost in the Conditioning Regimen for Autologous Hematopoietic Stem Cell Transplantation

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Introduction: High-dose melphalan plus autologous stem-cell transplantation as a consolidation therapy is an accepted treatment for multiple myeloma. Melphalan is an alkylating agent with most common side effects described as nausea and vomiting, mucositis and bone-marrow suppression. In Turkey, up to Social Health System's payment plan, original and generic (bioequivalent) melphalan could be prescribed for the patient. In this study, we aim to present the difference of original and biosimilar melphalan due to their toxicity profile and engraftment kinetics.

Materials (or patients) and methods: We retrospectively evaluated 39 patients (median age: 57 years, ranging 36-66 years) received high dose melphalan (200 mg/sqm) with autologous stem cell transplantation (ASCT) rescue between 2012-2014 at Ankara University Hematology Department. For conditioning regimen, 18 of 39 patients original brand melphalan, Alkeran (Glaxo-Smith Kline) were administered whereas other half used the equivalent form, Magvel(Windlas Biotech Limited®, India) ($n = 21$). For toxicitieswe compared the two groups by Pearson chi-square test. We used non-parametric Mann Whitney U testfor engraftment kinetics. $P < .05$ was considered statistically significant.

Results: The median age of patients in original melphalan group was 53 years (43-66 years), whereas the median ages in the generic group was 57years (36-64 years). The distributions of the genders were similar between the groups ($P = 0.4$). We did not observe any significant differences in the incidences of toxicities including diarrhea, oral mucositis and fever and also engraftment kinetics (Table 1) between two groups. The total parenteral nutrition needs were statistically similar in the groups. The cost of original melphalan was 400 €/per vial however generic ones were 37.9 €/per vial. There was no statistically difference detected in median hospital stay between groups.

Conclusion: Both original and generic (bioequivalent) form of melphalan had similar toxicity profile and engraftment kinetics. However, the generic form had markedly lower cost than original one. The main drawback of our study is that we did not evaluate the impacts of both agents on the responses of myeloma.

Disclosure of Interest: None declared.

P640

CALGB 100801 (Alliance): A phase II multi-center study of azacitidine (AZA) following allogeneic transplantation using a ''test dose'' targeted busulfan conditioning regimen for high risk myelodysplasia and older patients with acute myeloid leukemia

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Introduction: Relapse remains the major cause of death for patients with high risk MDS and older patients with AML undergoing stem cell transplantation using a reduced intensity conditioning regimen. Though conventional myeloablative conditioning is associated with significantly less relapse it is associated with increased toxicity especially in older individuals. We hypothesized that pharmacokinetic targeting to optimize busulfan (Bu) exposure a daily target Bu AUC level of 4000 uM*min based on a ''test dose'' strategy combined with the administration of AZA post transplantation would mitigate the risk of relapse with an acceptable non-relapse mortality (NRM) and thereby improve progression free survival (PFS).

Materials (or patients) and methods: We used this strategy as part of a RIC regimen on a prospective multi-center phase II trial conducted by the Alliance (formerly Cancer and Leukemia Group B (CALGB)). All patients were conditioned with a uniform regimen consisting of fludarabine IV (days -7 to -3), busulfan IV (Bu) targeted to a daily AUC of 4000uM*min (Days -6 to -3) following administration of a 25 mg/m2 test dose on one day between Days -14 to -9, and antithymocyte globulin (days -6, -5 and-4 (two doses for matched sibs and three for VUDs only). Beginning day + 42, all patients were planned to receive up to six monthly cycles of AZA at 32 mg/m2 subcutaneously x 5days. Eligibility included patients with AML in CR1 aged 60-74 years inclusive, MDS with IPSS risk $\geq$ Int-2 with less than 10% marrow blasts and age $<$75, availability of a well matched sibling or volunteer unrelated donor (VUD), and absence of significant end-organ damage prior to transplantation. The primary endpoint of the study was two year PFS. A secondary objective was to determine whether administration of the test dose of Bu with post test sampling would enable achievement of a daily target Bu AUC level of 4000 uM*min in at least 80% of the recipients.

Results: Sixty-eight patients were registered (Sept 2010-Oct 2013) and 64 underwent conditioning with 39 receiving grafts from VUDs and 25 from matched siblings. The median age was 63 (44-74), 20 had AML and 44 had MDS with IPSS low/int-1 14(32%), Int-2 22 (50%), High 5 (11%), missing 3(7%). Cytogenetics normal 29 (45%), complex 15 (23%), -7, del7q 7(11%), other 10 (16%), missing 3 (5%). The median AUC achieved on the validation sample was 4165 (2400-6642) uM*min. 87% (95% C.I 76-94) of patients were within 20% of target AUC based on validation sample. 42 patients started post transplant azacitidine. Maximum non-hematologic CTCAE v4.0 toxicity was grade 3 in 38 (59%), grade 4 in 6 (9%), and grade 5 in 14 (22%). There were ten deaths within the first 100 days after transplant; seven of these were due to NRM. With a median follow up of 703(42-1177) days, the estimated overall survival at 2 years for the entire population was 38% (95% C.I 0.26-0.54) for the AML cohort 49%(95% CI 0.29-0.82) and MDS 33% (95% CI 0.19-0.57).

Conclusion: In conclusion, results of this prospective multicenter trial suggest a strategy of targeting busulfan exposure to an AUC of 4000uM*min based on a prior ''test dose'' was successful in the majority of patients. Further follow up is necessary to determine whether this results in an improvement in PFS.
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P641
Efficacy of filgrastim biosimilar zarzio in autologous stem cells transplantation setting

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Introduction: Filgrastim is a human granulocyte colony stimulating factor (G-CSF) produced by recombinant DNA technology and is widely used for granulpoesis recovery in hematopoietic stem cells transplantations (HSCT). Neupogen (Amgen) is the original recombinant methionyl nonglycosylated human granulocyte colony stimulating factor (r-metHuG-CSF) and Zarzio (Sandoz) is filgrastim biosimilar approved in 2009 to have the same clinical effect as the original reference Neupogen. Daily practice clinical data are further needed to verify the safety and efficacy of biosilmilar products.
Materials (or patients) and methods: Observational single center study. Granulpoesis recovery in autologous HSCT patients was observed in filgrastim Zarzio treated group and was compared to historical control group of patients on Neupogen. Zarzio group characteristics observed in 10/2012-9/2014: $n = 148$ (female 47%), age 62 (24-71), multiple myeloma 65%, lymphoma 34%, others 1%, progressive/resistant disease 10%, chemotherapy HD-L-PAM200 48%, HD-L-PAM120 17%, BEAM 35%, the CD34 + number in the graft 5,6 (2,16-42,1) x10e6/kg. Neupogen group characteristics observed in 6/2009-9/2012: $n = 148$ (female 45%), age 60 (21-71), multiple myeloma 70%, lymphoma 26%, others 4%, progressive/resistant disease 7%, chemotherapy HD-L-PAM200 54%, HD-L-PAM120 16%, BEAM 30%, the CD34 + number in the graft 4,6 (1,97-34,9) x10e6/kg. The filgrastime dose was 5 μg/kg/day administered subcutaneously during neutropenia post-transplant (neutrophils 0,5x10e9/l) till neutrophils recovery (1,5x10e9/l). Basic statistical analyses were performed using statistical software (GraphPad InStat, GraphPad Software) with the Fisheŕs and Unpaired t-test. The $P < 0,05$ was considered as statistically significant difference.
Results: No statistically significant differences were observed between Zarzio vs. Neupogen group in respect to: age, gender, chemotherapy protocols, progressive/resistant disease, CD34 + number in graft, infections and FUO incidence (49% vs. 51%), FUO incidence (31% vs. 34%), non-relapse mortality till day + 100 (1,3% vs. 3%) and average number of filgrastim injections administered per a patient (6,2 vs. 6,4). The neutrophil engraftment count of 0,5x10e9/l and 1,0x10e9/l in Zarzio vs. Neupogen group was reached post-transplant on median day 11 (8-13) and 12 (9-14) vs. 11 (8-14) and 12 (9-14), respectively, $P = 0,02$. The average price per one Zarzio and Neupogen injection was 1110 CZK and 2840 CZK. No significant side effects, but skeletal pains in granulopoesis recovery, were observed both in 10% Zarzio and Neupogen treated patients.
Conclusion: Homogenous, large and comparable groups of patients were analyzed. Due to methodology characteristics of our observation, we are far to overestimate the statistically significant better recovery and lower number of injections observed in Zarzio group. We conclude, however, that Zarzio demonstrated similarity to the reference product Neupogen in respect to all observed variables, long term effects must be observed. Financial costs were significantly lower in Zarzio.
Disclosure of Interest: None declared.

P642
Alemtuzumab pre-conditioning prior to reduced intensity allogeneic transplant for T-cell Non Hodgkin Lymphoma is safe and feasible and may enhance the anti-lymphoma effect without increasing the rate of infectious complications

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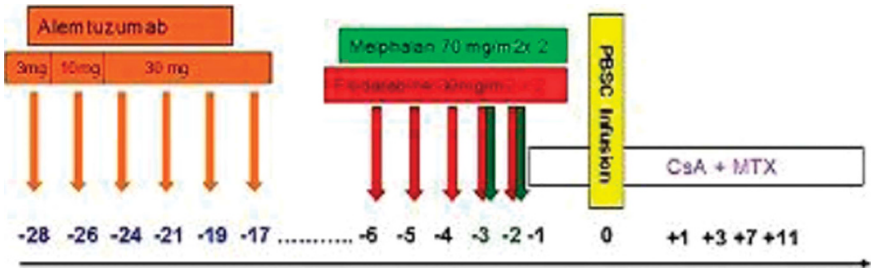
Introduction: In vivo T-cell depletion with Alemtuzumab reduces the incidence of acute and chronic graft-versus-host disease (GvHD) and graft failure. In addition, Alemtuzumab has considerable anti-lymphoma activity in T-cell neoplasms. However, it delays immune recovery and increases the risk of infectious complications.
We report on a pilot study analysing the safety and feasibility of Alemtuzumab as pre-conditioning prior to reduced intensity conditioning allogeneic stem cell transplantation (RIC-alloSCT) in patients diagnosed with T-cell non-Hodgkin lymphoma.
Materials (or patients) and methods: Six consecutive patients diagnosed with a T-cell lymphoid malignancy underwent RIC-alloSCT from a HLA-identical sibling ($n = 5$) or a matched unrelated donor ($n = 1$). Diagnosis were Angioimmunoblastic T-cell lymphoma ($n = 3$), hepato-splenic gamma-delta T-cell lymphoma ($n = 1$), T/NK cavum lymphoma ($n = 2$) and Sèzary Syndrome ($n = 1$). All received unmanipulated peripheral blood stem cells. At transplantation 4 patients were in PR, and 2 in CR. Median number of prior regimens was 2 (range 2-5), which included autologous stem cell transplantation in 3 cases. Conditioning regimen was based on Fludarabine and Melphalan in all cases. GvHD prophylaxis comprised Cyclosporine A and Methotrexate.
The pre-conditioning protocol consisted on 6 escalating doses of intravenous Alemtuzumab starting on day -28 (3 mg on day -28, 10 mg on day -26 followed by four doses of 30 mg on days -24 to -17) given 3 times a week (MWF) on an out-patient basis.
Results: Five patients received all six doses of Alemtuzumab as planned whereas one patient received only four doses for logistic reasons related to donor availability. Acetaminophen, Hydrocortisone and Dexchlorpheniramine were administered as pre-medication in all cases with no severe infusional reaction observed. There were no delays in proceeding to SCT conditioning and patients develop no infectious complications during the Alemtuzumab - alloSCT interval.
All patients engrafted with a median time to neutrophil engraftment of 23 days (range 14-27). All patients experienced delayed immune reconstitution (CD4 > 200/ul) at a median of 321 days (range 229-518). No opportunistic infection was observed apart from CMV reactivation developed in 3 out of 4 seropositive patients (no CMV disease was observed).
Acute skin GvHD (grade I and III) was observed in 2 patients (33.3%), both obtaining a CR with topical and systemic corticosteroids respectively.
Two patients developed mild ocular chronic GvHD (one after subsequent infusions of donor lymphocytes - DLI) but none has suffered from extensive chronic GvHD. All 4 patients in partial response prior to alloSCT achieved a complete remission. Only one patient (diagnosed with Sèzary Syndrome) experienced a relapsed and responded to DLI. With a median follow-up of 46 months, all patients remain alive and in CR.
Conclusion: Our preliminary results suggest that early administration of Alemtuzumab prior to reduced intensity conditioning is feasible and safe. In addition to providing T-cell depletion, Alemtuzumab may improve the response rate in T-cell lymphoid malignancies without increasing the risk of

[P642]



opportunistic infections. These results require further validation in larger phase II studies.
Disclosure of Interest: None declared.

P643
Clofarabine and Treosulfan as a safe conditioning for allogeneic haematopoietic stem cell transplant from matched donors: final results from a multicentric phase II study

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Introduction: Clofarabine is a new generation deoxyadenosine nucleoside analogue with documented both anti-leukemic and immune suppressive properties. The combination of Clofarabine and Treosulfan has been tested in a multicentric, open-label, non randomised phase II study ("Clotreo", EudraCT 2008-006972-31), aimed at evaluating its tolerability and efficacy in patients with haematological malignancies.
Materials (or patients) and methods: From November 2009 to November 2013, we enrolled 45 patients (median age 47 years), 37 affected by acute myeloid leukemia, 5 by acute lymphoblastic leukemia and 3 by myelodisplastic syndrome. 28 patients were "low-intermediate", while 17 were "high-very high" risk according to the Disease Risk Index (DRI) (*Armand et al, Blood 2012*). Conditioning regimen was based on: Clofarabine 40 mg/m^2 from day -6 to -2; Treosulfan 14 g/m^2 from day -6 to -4. Allogeneic peripheral blood- or marrow-derived haematopoietic stem cells from a sibling ($n = 23$) or a well matched unrelated donor ($n = 21$) were infused at day 0. Graft versus Host Disease (GvHD) prophylaxis was performed with Thymoglobuline at the dose of 1.5 or 2.5 mg/kg according to HLA match, Rituximab, Cyclosporine and short course of Methotrexate.
Results: The study is now closed, the last patient enrolled having reached the 1-year follow-up. Median follow-up among surviving patients is currently of 750 days (356-1716). Overall the regimen was well tolerated, the most frequent adverse events being body weight gain, skin/mucosal toxicity, transient renal impairment and liver enzymes alteration. The 2-year transplant related mortality rate was 17 + /-11% and did not increase in the long-term follow-up. Engraftment was fast with a median time to neutrophil and platelet recovery of 14 and 15 days respectively (10-27 and 11-156). All but one evaluable patients reached a full donor chimerism by day 30. The 2-year overall survival (OS) was 52%, with a significant difference when stratifying between patients with "low/intermediate" vs "high/very high" DRI (mean OS: 39 vs 8 months respectively, $P < 0.001$). 27% of patients experienced acute GvHD, with no differences between patients receiving a sibling vs an unrelated allo-transplant (25% vs 28% respectively). The 2-year chronic GvHD incidence was 23%. The 2-year relapse incidence was 61%, with patients belonging to the "low/intermediate" DRI group showing a significantly lower relapse rate as compared to the "high/very high" group (42% vs 100% respectively, $P < 0{,}001$). This directly affects the 2-year progression free survival: all patients = 30%, "low/intermediate" DRI = 47%, "high/very high" DRI = 0% ($P < 0{,}001$).
Conclusion: Treosulfan and Clorafabine combination is a feasible and safe conditioning prior to allogeneic hematopoietic stem cell transplantation (HSCT), and allows a prompt engraftment with rapid achievement of full donor chimerism. The considerable relapse incidence in patients with poor prognostic risk factors is still a major issue, and could be addressed through the modulation of *in vivo* T-cell depletion, possibly avoiding anti-thymocyte globulin administration in subjects with a "high/very high" DRI. Moreover, this combination is worth further clinical investigation in allogeneic HSCT setting focusing on patients with a disease sensitive to Clofarabine anti-tumor activity.
Disclosure of Interest: None declared.

P644
Allogeneic haematopoietic stem cell transplantation in children with acute myeloid leukemia: RIC versus MAC

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Introduction: The treatment of children with acute myeloid leukemia (AML) has been improved considerably over the past decades. Myeloablative conditioning regimens (MAC) are considered as standard conditioning therapy for allo-HSCT in children with AML. Reduced intensity conditioning (RIC) regimen is commonly applied to elderly patients and patients with comorbidities who are not eligible to MAC. Less data exist on RIC allo-HSCT in children. Some data suggest efficacy of allo-HSCT with RIC in children with ALL. The safety and efficacy of reduced-intensity conditioning (RIC) regimens for the treatment of children with AML is unknown.
The aim of this study was to estimate overall survival (OS) and of relapse rate after RIC vs MAC allo-HSCT in patients (pts) with AML, except of M3 FAB variant.
Materials (or patients) and methods: A total of 118 pediatric and adolescent patients with AML were included in to the study. The median age was 12.5 years (1-21), M/F distribution was 58 (49%) to 60 (51%). The median follow-up period was 27 (range 2-120) months. Fourty-two (36%) of the patients were in the 1st CR; 30 (25%), in the 2nd CR; 46 (39%) had relapsed disease. The patients received allo-HSCT from related ($n = 31$, 26%), or unrelated ($n = 78$, 74%) donor. The stem cell source was bone marrow in 59 (50%) patients and PBSC in 59 (50%) patients. In 66 patients (56%) MAC, and in 52 patients (44%) RIC were used. Acute GVHD prophylaxis consisted of cyclosporine A ($n = 79$, 67%), or tacrolimus ($n = 39$, 33%). Statistical analyses were performed using SPSS V17.
Results: Results of 10-year OS in depending of status disease at the moment of allo-HSCT and relapse rate of children with

AML after allo-HSCT (RIC versus MAC) are presented in Table 1

Table 1

parameters	*RIC*	*MAC*	P
10-year OS of pts in 1st CR	80% (95% CI; 59-99)	65% (95% CI; 45-85)	0,4
10-year OS of pts in 2nd CR	17% (95% CI; 1-37)	65% (95% CI; 35-95)	0,003
10-year OS of pts in active disease	19% (95% CI; 1-37)	17% (95% CI; 1-43)	0,4
Relapse rate	29% (95% CI; 12-48)	24% (95% CI; 10-41)	0,7

In patients receiving MAC or RIC allo-HSCT in 1st or 2nd CR the relapse rate did not correlate with donor type (related or unrelated, $P = 0.2$ for MAC and $P = 0.3$ for RIC) or stem cell source (BM or PBSC, $P = 0.9$ for MAC and $P = 0.4$ for RIC). There was also no correlation between relapse rate and patient's age: for patients under 14 years the OS rate was 20% (95% CI; 7-38) in MAC group versus 33% in RIC group (95% CI; 9-60) ($P = 0.5$); in patients of 14 to 21 years old, 29% in MAC (95% CI; 5-59) and 27% (95% CI; 5-56) in RIC group ($P = 0.9$). Allo-HSCT after RIC regimens was associated with significantly lower toxicity rates, while frequency of the infectious complications and GVHD (acute and chronic) was comparable for both MAC and RIC allo-HSCT recipients.

Conclusion: The efficacy of RIC and MAC allo-HSCT in generally is comparable for children with AML.

Disclosure of Interest: None declared.

P645

Excellent Outcome of Related Donor Hematopoietic Stem Cell Transplantation in Children with Fanconi Anemia Using Fludarabine-Based Conditioning Regimen: A Single Center Experience in TURKEY

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Introduction: Allogeneic hematopoetic stem cell transplantation(aHSCT) remains the only curative procedure for the correction of hematological abnormalities in FA patients. Studies demonstrated that regimen-related toxicity and high incidence of "graft vs host disease" (GVHD) were significant barriers to achieve success in these patients. It has been expected that fludarabine (Flu) based conditioning regimen for HLA-matched sibling donor transplants is to be better tolerated by patients with FA than the conditioning regimen with irradiation and alkylating agents as they exert cytotoxicity without direct DNA damage and with sufficient immunosupression.

Materials (or patients) and methods: We did a retrospective review of the 15 FAA patients who were transplanted in Ankara University School of Medicine, Pediatric BMT Unit between September 2004 and May 2014.

Results: Median age of the patients was 11.9 y (6.2 -16.5 y). All donors were HLA identical, 11 were siblings and 4 were family member. Stem cell sources were bone marrow (BM) in nine, peripheral blood (PB) in five, and BM plus PB in one patient. Preparative regimen consisting of fludarabine (150 mg/m^2/total) + cyclophosphamide (20-40 mg/kg/total) + anti-thymocyte globulin-Fresenius (32-36 mg/kg/total) was used in all patients. Ten patients received cyclosporine A (CsA) + methotrexate (8, 5 and 5 mg/m^2 on days +1, +3, +6, respectively), and the others received only CsA for GVHD prophylaxis. All the patients achieved both myeloid and thrombocyte engraftments. All patients were alive with normal hematological value at a median follow-up time of 77 months (range: 7-132 months). Twelve patients had complete donor chimerism and the other three patients had stable mixed donor chimerism (85-93%). Only one patient developed acute GVHD (grade II, isolated intestinal) and no patient had chronic GVHD. During follow-up time none of the patients developed secondary malignancy.

Conclusion: Based on our data, administration of lower doses of methotrexate for three days than the standard doses used in clinically practice, to be safe with minimal toxicities in the context of GVHD prevention in these patient group. We conclude that fludarabine based, non-irradiation conditioning regimen has favorable effect with low organ toxicity and stable engraftment in FA patients undergoing HSCT from matched related donors.

Disclosure of Interest: None declared.

P646

FLAMSA reduced-intensity regimen in high-risk AML patients – role of re-induction and disease state prior to transplant

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Introduction: Reduced intensity conditioning (RIC) regimens as compared to myeloablative protocols have demonstrated lower toxicity profiles at similar efficacy in the context of allogeneic haematopoietic stem cell transplantation (allo-SCT) for patients with acute myelogenous leukaemia (AML) in first or second remission. In addition, the FLAMSA RIC regimen, combining a cytoreductive part and a transplant-conditioning part, has been described to be efficacious in patients with refractory disease. Re-induction treatment after AML relapse or refractory disease is often accompanied by severe, in particular, infectious complications such as fungal pneumonia caused by long neutropenic phases, precluding a significant proportion of patients from completing allo-SCT. Aim of this study was to analyse the role of re-induction therapy prior to transplant.

Materials (or patients) and methods: We retrospectively analysed clinical data of 118 consecutive patients with high-risk primary or relapsed primary AML after allogeneic stem cell tranplantation following FLAMSA conditioning at our center. No prophylactic donor lymphocyte infusions were administered.

Results: Median age of the cohort was 53 years (19-73 years). Donors were matched related, matched unrelated or mismatched unrelated in 33, 49 and 18% of the transplants. Complete remission prior to transplant was detected in 29% and 21% of the patients after induction or re-induction, respectively. Twenty-five percent of the patients were transplanted with blast persistence, both in the group of patients after first induction and re-induction treatment. Median follow-up was 27 months.

The 4-year overall survival of the whole cohort is 44% with a 4-year relapse-free survival of 42%. Cumulative incidence of relapse was 26, 30 and 40% at one, two and four years, respectively. Cumulative incidence of non-relapse mortality (NRM) was 15, 18 and 18% at one, two and four years, respectively.

There were no significant differences regarding overall and relapse-free survival for patients transplanted in CR1, CR2 or blast persistence after induction treatment. Patients who failed remission after re-induction therapy showed significantly worse overall and relapse-free survival ($P = 0{,}049$ and 0,003, repectively, log-rank test). NRM was similar in all cohorts.

Conclusion: FLAMSA is a highly effective conditioning regimen for high-risk AML patients even not in CR. Thus, the decision for re-induction therapy prior to allogeneic SCT has to be weighed against the potential toxicity of this approach.

Disclosure of Interest: None declared.

P647
FEAM conditioning in Non Hodgkin/Hodgkin relapsed or refractory Lymphomas

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Introduction: High-dose therapy with autologous stem cell transplantation (ASCT) has been widely proposed for patients with relapsed non Hodgkin/Hodgkin lymphoma (NHL/HL). One of the most commonly used conditioning regimen is fotemustine plus etoposide, cytarabine and melphalan conditioning (FEAM) that lately in some cases has replaced conditioning with carmustine etoposide cytarabine and melphalan (BEAM). Still, limited data is available concerning the management of this regimen, the patient outcome and toxicity levels. The reason by which fotomustine is used in place of carmustine is due to the fact that studies conducted on mice and humans have demonstrated that this nitrosourea alkylating agent has a better toxic profile regarding lungs and liver toxicities. Also, unlike carmustine, the lipophilic nature of fotoemustine allows it to cross the blood brain barrier exhibiting an additional application on the central nervous system.
Here we present a two-center study with the aim of investigating patients' outcomes when treated with FEAM conditioning followed by ASCT.
Materials (or patients) and methods: We collected 78 patients who underwent FEAM conditioning followed by ASCT. Patient characteristics at diagnosis were: sex ratio (M/F): 1.3; median age: 48 years old (range 21-75). We considered the stage at diagnosis (stage I-II: 24 patients, stage III-IV: 54 patients), central nervous system (CNS) ($N = 7$) and bone marrow involvement ($N = 21$). In terms of histology, we composed the following subgroups: 61 NHL divided into 50 aggressive NHL (Diffuse Large B Cell Lymphoma, Burkitt Lymphoma and Mantle Cell Lymphoma) and 11 indolent NHL (Follicular lymphoma and Marginal Zone Lymphoma), and 17 HL. Patients' response after first-line therapy was divided as follows: 30 complete remissions, 42 partial response and 6 relapsed/progression disease. Patients who achieved CR subsequently relapsed. All patients underwent salvage chemotherapy; after this, 41 patients achieved a CR, 33 achieved a PR and one patient developed PD. The other three patients were lost to follow-up. The median interval between diagnosis and high-dose therapy was 48,5 months. After ASCT, patients were subjected to close clinical evaluation, complete blood counts and serum chemistries to establish and monitor FEAM toxicities and median time to engraftment. After a median time of six months, patients underwent complete restaging to estimate clinical response to high dose conditioning.
Results: Median time to neutrophil ($>$ 500/mcl) and platelets recovery ($>$20000/mcl) were 12 and 9 days, respectively. As regards mucositis, 26 patients developed G3 oral mucositis and 3 of them G4 mucositis; G3 diarrhea occurred in only one patient while none of them experienced G3/G4 chemotherapy-induced nausea and vomiting. We didn't observe major liver, pulmonary or kidney events. Considering patients' status at the last follow up, of the 75 patients who underwent FEAM followed by ASCT, 46 had a CR, 8 had a PR, 3 of them experienced a PD, 1 was classified as RD and 17 died.
Conclusion: This study not only confirms the feasibility and low toxicity of FEAM regimen, but adds something about its effectiveness on maintaining deep response and converting partial into complete response.Finally, none of our patients developed, during follow-up, CNS sanctuary involvement. This could be explained by fotoemustine overcoming of the blood brain barrier.
Disclosure of Interest: None declared.

P648
Comparison of Intravenous and Oral Busulfan in Allogeneic Hematopoietic Stem Cells Transplantation in Acute Leukemias

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Introduction: The regime most frequent used as conditioning in Allogeneic Hematopoietic Stem Cells (HSCT) is Busulfan (BU) and Cyclophosphamide (BUCY) in acute leukemias, initially Busulfan was introduced orally, but since the implementation of its intravenous (IV) form has been observed a decrease in adverse effects with very good results, so the aim of this study is to compare BU IV and PO in conditioning allogeneic transplantation CPH at the National Cancer Institute of Mexico City.
Materials (or patients) and methods: A comparative retrospective study from January 2011 to November 2014, in patients with the diagnosis of acute leukemia (myeloid or lymphoid) who received Allogeneic HSC with BUCY Conditioning, aged 15 years, who received SC of peripheral blood, HLA identical. Patients younger than 15 years, those who received autologous transplantation, chronic leukemia and other storage protocols were excluded. A sample of 45 patients, of which one group received oral BU ($n = 22$) and the other group receiving BU IV ($n = 23$), which follows administaron oral BU 1 mg/kg every 6 hours was for 16 doses (days -7 to -4), BU IV 0.8 mg/Kg every 6 hours for 16 doses (days -7 to -4), in both cases followed Cy 60 mg/kg/day for 2 days (day -3 and -2) and mesna 60 mg/kg/day for 2 days, not received anticonvulsant prophylaxis. An analysis of dichotomous variables was performed, the difference in treatment was assessed in groups with the chi-quare test, for data analysis the statistical program STATA version 12.1 was used was evaluated. This research was approved by the ethics committee of the institution.
Results: The average age of patients was 28.6 years, with a range of 15-55 (S.D. 10.9 years), 44.5% were women ($n = 20$), 55.6% males ($n = 25$). 87.5% of patients who received oral BU had severe mucositis (x^2:5.8; d.f. $= 1$, $P < 0.05$), while 12.5% with BU IV for mortality 59.1% (x^2:9.05; d.f. $= 2$, $P < 0.01$) patients died in the first group, had lower mortality rate in the second group. None had Sinusoidal Obstruction Syndrome (SOS).
Conclusion: We found a significant difference in the use of oral and intravenous Busulfan regarding the severity of mucositis, it is less common with intravenous form, probably by a decrease in toxicity. Higher mortality was found before 12 months in the group receiving oral BU. One limitation of the study was that the patient sample is small, so we suggest more studies with a larger sample of patients.
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Disclosure of Interest: None declared.

P649
HLA-haploidentical hematopoietic stem cell transplantation with post-transplantation cyclophosphamide in a child with sickle cell disease

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) offers the possibility of permanent cure for sickle

cell disease (SCD) patients thereby avoiding considerable morbidity, long-term complications and decreased life expectancy. Unfortunately, the probability of finding an HLA-matched donor are limited. In SCD patients, HSCT from haploidentical donors using reduced intensity conditioning, T-cell replete marrow and post-grafting immunosuppression with cyclophosphamide has resulted in negligible toxicity but high rates of graft rejection (Bolaños-Meade, Blood, 2012).

Materials (or patients) and methods: We report the case of an 8 year old boy with several disease-related complications such as acute thoracic syndrome and recurrent pain crises despite treatment with hydroxyurea. He further suffered from osteomyelitis, radiologic signs of cerebral vasculopathy and silent strokes without neurologic impairment. No HLA-identical sibling or matched unrelated donor were available.

He therefore underwent HLA-haploidentical HSCT from his father using un-manipulated bone marrow as graft (5.7x10^8 TNC/kg). Myeloablative conditioning consisted of alemtuzumab 0,2 mg/kg/d (days -9 to -8), treosulfan 14 g/m^2/d (days -7 to -5), fludarabine 30 mg/m^2/d (days -7 to -3), thiotepa 2x5mg/kg/d (day -4) and cyclophosphamide 14,5 mg/kg/d (days -3 to -2). GvHD-prophylaxis was performed using cyclophosphamide 50 mg/kg/d on days +3 and +4, mycophenolate mofetil and tacrolimus from day +5. Before conditioning, partial exchange transfusions were performed to decrease the HbS level to <30% in order to prevent SCD related complications during the transplant period.

Results: The patient experienced minimal acute toxicity (mild mucositis). Low level, asymptomatic CMV viremia was treated with ganciclovir. Engraftment occurred on day +12 (neutrophils) and +14 (platelets) respectively. No red blood cell transfusions were neccessary. No GVHD has been observed at 110 days of follow-up with 100% peripheral blood donor chimerism on day +100.

Conclusion: Haploidentical HSCT can extend the donor pool for patients with SCD. Previous protocols using post-transplantation cyclophosphamide lead to low levels of transplantation associated mortality and GvHD in SCD. Intensification of the conditioning regimen may lead to improvement in engraftment rates while still offering low toxicity.

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Disclosure of Interest: None declared.

P650

Unique Chemotherapy Based Reduced Intensity Conditioning Regimen for Patients Transplanted from HLA Identical Siblings, Haploidentical or Volunteer Unrelated Donors: a Prospective Study from the Rome Transplant Network

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Introduction: Reduced Intensity Conditioning (RIC) regimen, by decreasing early transplant related mortality (TRM) and morbidity, allows allogeneic hematopoietic stem cell transplant in older or unfit patients. Several different regimens have been used, most of them including low dose TBI. Herein, we report outcomes of patients with high-risk haematological malignancies transplanted from HLA Identical Siblings (Id-Sibs), Haploidentical (Haplo) or Volunteer Unrelated Donors (VUD) according with the policy of the Rome Transplant Network (RTN), which considers a unique chemotherapy based RIC for all patients older than 55 years or unfit for a myeloablative conditioning regimen.

Materials (or patients) and methods: From April 2008, 74 consecutive patients with a median age of 57 yrs (range, 22-66; 66% > 55 yrs) underwent RIC-HSCT for acute leukemias (AML = 34; ALL = 8), chronic lymphoid (NHL = 9; HD = 7; MM = 3; CLL = 4) and chronic myeloid (MDS = 6; CML = 3) disorders. The stem cell source was Id-Sib (31%), VUD (31%) and Haplo (38%) donors. At time of transplant, 35 patients were in early (1st or 2nd CR) and 39 in advanced (>2nd CR or active disease) disease stage. Patient characteristics were homogeneously distributed between the three stem cell sources. A unique RIC regimen (TBF) consisting of Thiotepa (5 mg/kg on day -6), Busilvex (3.2 mg/kg on day -4 and -3) given as single fraction over 3 hours infusion and Fludarabine (50 mg/m^2 on day -5, -4, -3) was used. The GvHD prophylaxis consisted of the classic association of MTX + CSA for all patients. In addition to MTX and CSA, VUD patients received antithymocyte serum (ATG), while GvHD prophylaxis was intensified with ATG, MMF and Basiliximab, an anti CD25 monoclonal antibody, in patients undergoing an unmanipulated bone marrow transplant from haploidentical donor.

Results: Overall, with a median time of 17 days, the cumulative incidence (CI) of PMN engraftment was 96 ± 2% with hematopoietic reconstitution of donor origin in any case. The CI of III - IV grade acute GvHD was 8 ± 3%. The 1- and 5-year CI of TRM was 21 ± 3% and 30 ± 3%, and of relapse 33 ± 4% and 50 ± 4%, respectively. For these outcomes no statistical differences were observed between the three donor sources (Id-Sibs vs VUD vs Haplo). The median follow-up was 30 months from transplant. The results of 5-years overall and disease-free survival probabilities are listed in the table.

	All	*Id-Sibs*	*VUD*	*Haplo*	P
OS, %	42 ± 7	54 ± 12	58 ± 11	22 ± 9	0.035
DFS, %	24 ± 6	39 ± 12	19 ± 10	15 ± 8	ns

Conclusion: RIC-TBF combined with the respective GVHD prophylaxis regimen guarantees engraftment in either Id-Sib or VUD or Haplo transplant with low incidence of acute GvHD. Taking in account the high clinical and hematological risk of the patient series, the TRM can be considered acceptable, but relapse rate remains high in all patient categories. Finally, independently from the available stem cell sources, RIC-TBF offers a high probability of long-term survival in frail patients candidate to an allogeneic transplant.

Disclosure of Interest: None declared.

P651

Low-dose decitabine combined with modified BUCY as conditioning regiment follow by allogeneic stem cell transplantation for the treatment of advanced AML/MDS patient

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Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment options to hematologic malignancies. However, majority of patients with refractory or resistant hematologic malignancies can not achieve remission before transplantation. It is necessary to design a safe and affective conditioning regimen to reduce the tumor burden, improve the remission rate, decrease the transplantation related mortality and improve disease-free survival in patients with advanced acute myloid leukemia(AML) and myelodysplastic syndrom(MDS). One of the promising drugs of epigenetics is decitabine (DAC), which has a significant effect on a variety of hematologic malignancies including MDS and advanced AML. Furthermore, decitabine

can not only up-modulate the tumor-associated antigen express on surface of leukemia cells to increase graft-versus-leukemia (GVL) effect but also can reduce the incidence of graft-versus-host disease (GVHD) by increase the number of regulatory T Cells (Tregs).
Therefore, this clinical study will investigate the security and efficacy of conditioning regimen containing low-dose decitabine combined with modified BUCY regimen for advanced AML/MDS patients and explore the role of immunomodulatory activity post transplantation.
Materials (or patients) and methods: 20 cases of patients with advanced AML/MDS underwent allo-HSCT with low-dose decitabine (integral dose 100 mg/m^2) combined with modified BUCY conditioning regimens.
Results: 19/20 (95%) patients achieved complete remission and hematopoietic reconstitution after transplantation.The median time of neutrophil and platelet recovery were 12 (10-22) and 14.5 (12-35) days respectively. The transplantation-related mortality (TRM) rate was 0. The cumulative rate of aGVHD and cGVHD were 25.6% and 48.3%.And the cumulative rate of aGVHD for grade III and IV were 10.6%. The cumulative relapse rate was 26.7%. During a median follow-up of 246 (19-613) days, 15 patients were disease free survival.The estimated 2-year overall survival (2 yr-OS) rate was 78%. The 2 year disease-free survival (2 yr-DFS) rate was 62.6%. Furthermore, There was no significant difference of the estimated 2- yr OS and DFS between patients with or without DNMT3A mutations or abnormalities of chromosome 7 and complex chromosomal karyotype.
Conclusion: 1.It is feasible to use conditioning regimen containing low-dose decitabine combined with modified BUCY regimen before allo-HSCT.The treatment were well tolerated and transplantation-related mortality (TRM) rate was 0. 2.The incidence of aGVHD and cGVHD were not increased in this setting. 3. 92.3% patients achieved complete remission with salvage allo-HSCT with ralative high 2- yr rate of OS and DFS. 4.The prognosis and survival of the patients with complex chromosomal karyotype or chromosome 7 abnormalities or DNMT3A mutation may be improved by treating with decitabine containing conditioning regimen.
Disclosure of Interest: None declared.

P652
Conditioning Including Low-Dose Busulfan, Low-Dose Cyclophosphamide, and Fludarabine for Unrelated and Haploidentical Donor Stem Cell Transplants in Severe Aplastic Anemia

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Introduction: Clinical outcomes of alternative donor hematopoietic stem cell transplantation (HSCT) for severe aplastic anemia (SAA) have been improved with the conditioning including fludarabine, low-dose cyclophosphamide, and antithymocyte globulin (ATG). But graft rejection rate in unrelated transplant (URT) is around 15-17%. We designed a conditioning including low-dose busulfan, low-dose cyclophosphamide, and fludarabine for both URT and haploidentical-HSCT (haplo-HSCT) in SAA in order to reduce graft rejection and improve survival. **Objective:** In present study, the safety and efficacy of a novel conditioning containing low-dose busulfan, low-dose cyclophosphamide, and fludarabine for URT and haplo-HSCT in SAA were evaluated.
Materials (or patients) and methods: Between September 2012 and July 2014, total 34 patients with SAA who received either URT or haplo-HSCT in our center were analyzed retrospectively. Donor matching was performed for HLA-A, -B, -C, DRB1 and DQB1 using high-resolution DNA typing. The source of stem cells was either peripheral blood (PB) for URT or a combination of bone marrow (BM) and PB for haplo-HSCT. Conditioning consisted of busulfan (3.2 mg/kg per day) for 2 days; fludarabine (30 mg/m^2 per day) for 4 days; cyclophosphamide (500 mg/m^2 per day) for 4 days; and either ATG (Thymoglobuline, SANOFI, total dose 7.5 mg/kg in 9 cases; ATG-F, total dose 20 mg/kg in 16 cases) or alemtuzumab (total dose 1 mg/kg in 9 cases). For GVHD prophylaxis, CsA/tacrolimus, MMF and MTX were used in patients received ATG; CsA/tacrolimas alone was used in patients received alemtuzumab. Chimerism for BM and CD3 cells was evaluated
Results: The median age was 15 (2-60) years. Male to female was 15:19. The median disease course from diagnosis to HSCT was 15 (2-132) months. 12/34 (35.3%) patients received one course of ATG before transplant. 23/34(67.6%) cases had heavy transfusion. Two patients were diagnosed as SAA-PNH. Seventeen patients underwent URT and 17 patients received haplo-HSCT. One of 34 (2.9%) patient (URT) had primary engraft failure and achieved durable engraftment after 2nd transplant (haplo-HSCT). 33/34 (97.1%) patients achieved full donor chimerism by $+30$ days post-HSCT. No secondary graft rejection occurred. The median time to neutrophil engraftment was 14 (9-19) days; the median time to platelet engraftment was 12 (6-42) days. With a median follow-up 9 (2-26) months, one-year overall survival (OS) was 91.2%. One-year OS rates were 94.1%, 84.4% in URT, haplo-HSCT, respectively ($P = 0.531$). Three of 34 (8.8%) patients developed grade III-IV aGVHD. Six cases had cGVHD (2 in limited, 4 in extensive). Three patients died at $+42$, $+180$, $+240$ days due to mucormucosis, intracerebral hemorrhage, and EBV pneumonia, respectively.
Conclusion: Our results have shown that favorable outcomes of both URT and haplo-HSCT have been achieved with this novel conditioning, which has lower graft rejection and better survival even in patients with long disease course and heavy transfusion. Haplo-HSCT in SAA has obtained comparable outcome with URT; therefore, haploidentical family member is an important alternative option for HSCT in patients with SAA.
Disclosure of Interest: None declared.

P653
Should all the pediatric HSCT patients benefit from busulfan therapeutic drug monitoring? Experience on a large cohort

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Introduction: Busulfan (Bu) used prior to hematopoietic stem cell transplantation (HSCT) presents a narrow therapeutic index: low drug exposure is associated with increased risk of graft rejection or relapse, and high drug exposure with toxicity (in particular veno-occlusive disease VOD). Therapeutic drug monitoring (TDM) and dose adjustment to achieve a target of area under the concentration-time curve (AUC) of 900 to 1500 μM.min has been shown to improve the outcome. The aim of this study was to assess the impact of Bu TDM in a large cohort of children.
Materials (or patients) and methods: One hundred forty-two HSCT corresponding to 138 patients receiving Bu 4 times a day for 4 days were analyzed. The first dose was adjusted to the patients' body weight (BW) as recommended by Bu manufacturer. Two blood samples were drawn 2.5 h and 4.5 h after the first dose. Afterwards, Bu PK parameter values (one-compartment model) and AUC were estimated by using Bayesian estimation implemented in the USC-PACK software and were used to predict future Bu plasma levels, subsequent individual AUC and to calculate the doses needed to reach the target AUC range. The main clinical endpoints were engraftment (full engraftment if donor chimerism $\geq 95\%$) and occurrence of VOD. Event-free survival (EFS) and overall survival (OS) after HSCT were evaluated for patients with a follow up of at least 6 months.

Results: The median follow-up was 54 months (range, 9–104). Mean age was 6.5 years (range, 0.2-21), mean weight was 23.6 kg (3.2-87), and 23 patients weighted less than 9 kg. Fifty-one percent of children had a hematological malignant disease, 21% had non-malignant tumors, 12% had solid malignant tumors and 16% had hemoglobinopathies. One hundred twenty-five patients received allogeneic HSCT (88%), 17 received autologous HSCT (12%).
Bu AUC were within the AUC target range in 68% of children after the first dose and the mean predicted AUC for the 16 doses would have been in the target range in 74.8% of children if the initial dose was maintained. Bu dose adjustment was performed in 54.9% of patients, whatever were the weight and pathology. At least one dose adjustment higher than 30% was necessary in each weight and pathology group. Dose individualization increased the probability to achieve the AUC target to 91% after the 16 doses ($P < 0.001$). Achieving the target in thalassemia patients (75%) was less easy than other (92.3%, $P = 0.047$). Full engraftment occurred in 80.1% of children, while 4.3% experienced rejection. Overall EFS was 88.5% and OS was 92%. VOD occurred in 26 patients (18.3%), none of whom showed a Bu AUC less than 1500 μM.min. In multivariate Cox regression analysis, BW < 9 kg was found to influence the onset of VOD. No correlation was found between Bu AUC and VOD occurrence.
Conclusion: This study confirms the importance of TDM and PK-based dose individualization for safe and effective Bu use prior to HSCT for all children and not only a restricted population as usually done. However, further research is necessary to identify a PK criterion linked more specifically than AUC with VOD.
Disclosure of Interest: None declared.

P654
Fludarabine/TBI conditioning for elderly patients with acute leukaemia/MDS: low toxicity and low relapse

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Introduction: The Fludarabine/TBI (Seattle) protocol is a non-myeloablative conditioning regimen which is commonly used for HSCT in patients with multiple myeloma or chronic lymphocytic leukaemia. In the UK this regimen is used less commonly in patients with acute leukaemia but may be better tolerated by elderly patients or those with co-morbidities. We report our single centre experience of using Flu/TBI conditioning for patients undergoing allogeneic transplant for acute leukaemia or myelodysplasia.
Materials (or patients) and methods: A retrospective review of the medical records of all patients undergoing HSCT for acute leukaemia or MDS who received Flu/TBI conditioning was undertaken. Permission for data collection as part of a service evaluation was obtained from the Manchester Royal Infirmary audit committee.
Results: Fifteen patients underwent HSCT with Flu/TBI conditioning between Sep 2012 – Jan 2014. 11/15 (73%) had AML, 1/15 had ALL (7%) and 3 /15 had MDS (20%). 7/15 (47%) had normal cytogenetics. The remaining 8/15 (53%) had the following cytogenetic abnormalities: 5q & del 13q, trisomy 8, inversion 11, trisomy 11 & 14, del 20q, t (9;22), 7q abnormality. The median age was 65 years (range 54–74 years). There were 10 males and 5 females. One patient had a sibling donor and 14/15 had unrelated donors (10/10 match in 11 patients and 9/10 in 4 patients). The CMV status was pos/pos for eleven and neg/neg for four. The median HCT comorbidity index was 2 (range 0-7). Median length of hospital stay was 12 days (range 6-31). 67% (10/15) patients had an uneventful admission, 6.5% developed conjunctivitis, 6.5% had fluid overload and 20% developed neutropaenic fever. There were no HDU/ITU admissions. Only 27% (4/15) suffered CMV reactivation. There were no cases of acute GvHD in our cohort and 73% of patients developed chronic GvHD. 4 patients had severe chronic GVHD on NIH criteria. There was a 60% (9/15) readmission rate after initial discharge. 55% (5/15) were re-admittedonce, 1/15 was re-admitted 3 times and 1/15 was re-admitted 4 times. The reasons for re-admission were lower respiratory tract infection 22%, gastroenteritis 11%, CMV reactivation 22%, nutritional support 11%, neutropaenic fever 22% and GvHD 11%. At a median follow up of 13 months (range 6 to 23) one patient has died. The cause of death was GVHD. 2/15 (13%) of patients have relapsed. 13/15 (87%) of patients reached 100% PBL donor chimerism and 60% of patients reached 100% CD3 + donor chimerism at some time point. Of those reaching 100% PBL chimerism the median time elapsed was 184 days and of those reaching 100% CD3 + chimerism the median time elapsed was 215 days.
Conclusion: Allogeneic HSCT with non-myeloablative fludarabine/TBI conditioning in patients with MDS/AML may be a feasible alternative to standard reduced intensity regimens in older patients or patients with high comorbidity indexes. The high percentage of uneventful admissions suggests that HSCT could be carefully carried out on an outpatient basis. The high incidence of chronic GVHD and low relapse rate suggests a successful graft-versus-leukaemia effect in this high risk group. Further prospective studies are required to determine the role of Fludarabine/TBI conditioning in elderly patients undergoing HSCT for AML/MDS.
Disclosure of Interest: None declared.

Minimal residual disease, tolerance, chimerism and immune reconstitution II

P655
WT1 and flow cytometry Minimal Residual disease follow-up after allogeneic transplantation in practice

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Introduction: Post allogeneic hematopoietic stem cell-transplantation (alloHSCT) relapse remains the first cause of death in acute myeloid leukemia (AML) transplanted patients. Minimal residual disease (MRD) assessment by Multiparameter flow cytometry (MFC) or WT1 expression level have been proposed to predict relapse in AML patients before and after alloHSCT. We compared MRD levels using MFC or WT1 for the prediction of AML relapse after alloHSCT.
Materials (or patients) and methods: We retrospectively analyzed all patients who received an alloHSCT for AML between January 2003 and December 2013. MRD was assessed by MFC and WT1 30 days before and 30, 60, 100 and200 days after alloHSCT. Sensitivity, positive and negative predictive value and the delay between MRD positivity and cytological relapse were calculated at each time.
Results: One hundred and three patients received an alloHSCT for AML between 2003 and 2013. Sixty-nine patients (67%) were excluded because they lacked WT1-MRD follow-up. Among the 34 remaining patients, 30 (88%) had reached criteria for a cytological medullar complete response (70% in first CR, 18% in second CR or more). Reduced intensity conditioning was performed in 18 (52.9%) patients, myeloablative conditioning in 15 (44.1%) and sequential in 1 (2.9%). The median follow up was 1.12 years [95%CI: 0.6982-1.624]. Thirty four (33%) had an evaluable MRD. Eight (24%) patients relapsed, with a median of 116 days after alloHSCT [95%CI: 50-427 days] and 4 (12%) died of a non-relapse cause, with a median of 82 days post alloHSCT [95%CI: 56-286 days]. MRD evaluation by MFC and WT1 predicted relapse with a good sensitivity (Se = 0,67 at day 100 and 1 at D200 for WT1,

Se = 0,56 at D100 and 1 at D200 for MFC) and negative predictive value (NPV = 0,88 at day 100 and 1 at D200 for WT1, NPV = 0,85 at D100 and 1 at D200 for MFC) at D100 and D200 after alloHSCT. We failed to predict relapse at other time points. However, even at D100 and D200, the delay between MRD positivity and relapse was too short to allow any therapeutic intervention (Median 0 days [95%CI:0-29] for MFC and WT1). MRD measurement by MFC and WT1 was well correlated at D100 and D200 time points (R2 = 0,59 for D100 and 0,67 for D200). In multivariate analysis, DLI and GvHd did not influence relapse relative risk.

Conclusion: MRD assessment is comparable between WT1 and MFC, but the delay between MRD positivity and cytologic relapse is too short to be relevant. This data remain to be validated on a prospective cohort.

Disclosure of Interest: None declared.

P656

Quantitative PCR is a sensitive and reliable tool for chimerism monitoring in peripheral blood and bone marrow after unrelated HCT

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Introduction: Although short tandem repeat (STR) analysis has long been considered as the gold standard for monitoring of donor-recipient hematopoietic chimerism (HC) after allogeneic hematopoietic cell transplantation (HCT), interest in methods based on quantitative PCR (qPCR) for this purpose is increasing, due to the higher sensitivity of the latter approach.

Materials (or patients) and methods: We have performed a retrospective analysis of donor-recipient HC in a cohort of 30 patients who underwent HCT from a 10/10 or 9/10 HLA-matched unrelated donor at the Department of Bone Marrow Transplantation at the University Hospital Duisburg-Essen, between the years 2006 and 2013, for the cure of AML, ALL, MDS or CML. A total number of 493 follow-up samples, including 384 peripheral blood (PB) and 109 bone marrow (BM), were tested for HC in parallel by STR or by a commercial insertion-deletion qPCR (AlleleSEQR Abbott Molecular). Based on recent publications (*Willasch et al., Biol Blood Marrow Transplant 2014; Qin et al. Bone Marrow Transplant 2014*), the threshold for positivity in qPCR HC was set as 0.1% for PB and 1% for BM.

Results: For all 30 donor-recipient pairs, at least three informative markers (i.e. positive in the patient but negative in the donor) were available in the total set of 34 markers, with a median of 7 markers per patient. 2 markers were selected for follow-up of each patient, and showed high inter-marker reproducibility ($r^2 = 0.98$). 45 samples drawn in parallel from BM or PB showed high levels of concordance between the absolute percentages of qPCR HC obtained for the two sources ($r^2 = 0.98$). In 36 BM samples from 14 patients who maintained a stable complete chimerism (CC) in STR after transplantation, qPCR HC never exceeded the 1% threshold for positivity in BM, resulting in 100% specificity. In contrast, qPCR HC exceeding the 0.1% threshold for positivity in PB was found in 12/189 PB samples from patients with CC, resulting in a specificity of 93.6%. Relapse prediction could be investigated in 9 patients for whom at least 2 PB or 1 BM sample was available prior to relapse, and showed a sensitivity of 77.8% and 50% for qPCR on PB and BM, respectively, compared to 11.1% and 30%, respectively, for STR.

Conclusion: We conclude that HC analysis by qPCR is feasible after unrelated HCT, both on PB and BM samples, and appears to present an appealing degree of sensitivity for relapse which is only partly traded off by decreased specificity. Prospective studies to further investigate the potential of qPCR HC for post-transplant monitoring especially of high risk patients are warranted.

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P657

Th17, Tc17 and Tregs in pediatric allogeneic stem cell transplantation: associations with Interleukin-7 and aGvHD

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Introduction: IL-7 is essential for *de novo* T cell generation in thymus and peripheral T cell homeostasis. Our previous studies indicate that IL-7 receptor polymorphisms and high plasma levels of IL-7 in the early post-transplant phase are associated with inferior T cell reconstitution and increased risk of aGVHD and TRM. However, little is known regarding associations between IL-7 levels and the profile of functionally different T cell subsets.

We investigated reconstitution of pro-inflammatory Th17 and Tc17 cells along with Tregs in the post-transplant phase, and analyzed associations with IL-7 and aGvHD.

Materials (or patients) and methods: We included 34 children undergoing allogeneic HSCT at Rigshospitalet, Denmark, from 2010-2013. Median age was 7.9 years (range 1.2-15.8 years). Diagnoses included malignant ($n = 20$) and benign diseases ($n = 14$). Donors were either SIB ($n = 6$), MUD ($n = 24$) (BM or PBSC grafts) or unrelated UCB grafts ($n = 4$). Conditioning regimens included TBI ($n = 9$), high-dose chemotherapy ($n = 16$) or were fludarabine-based ($n = 9$). The 28 patients transplanted with an unrelated donor received additional ATG.

T cell subsets were counted by flow cytometry day +90 and +180 and defined as follows; CD4+ T cells, CD8+ T cells, CD4+CD161+CD196+ Th17 cells, CD8+CD161+CD196+ Tc17 cells and CD4+$CD25^{high}CD127^{low}$ Tregs. T cell subsets were analyzed for expression of the naivety T cell marker CCR7 and the effector T cell markers CXCR3 and GARP.

Plasma IL-7 was measured by ELISA (R&D Systems) at day -14, 7, 14, 21, 28, 60 and 90 post-transplant.

Results: All T cell counts increased from day +90 to day +180, although most pronounced for Tregs ($P = 0.0067$), with

a corresponding decrease in the CD4+ Th17/Treg ratio ($P = 0.060$). Expression of CCR7 was significantly higher in CD4+ T cell populations than in CD8+ T cell populations (MFI: 341 in CD4+ T cells vs. 106 in CD8+ T cells day +90, $P < 0.0001$), while the expression of CXCR3 and GARP was generally low.
Cell dose correlated positively with all T cell subset recoveries. Non-malignant diagnosis was associated with increased expression of CCR7 on CD4+ and Th17 cells.
IL-7 levels increased post-HSCT, reaching a maximum at day +7, followed by a gradual decline. High IL-7 levels at day +7 and +90 were associated with reduced numbers and less naive CD4+ T cells ($P = 0.0034$, $\beta = -22.9 \times 10^6$ cells/L), CD8+ T cells ($P = 0.013$, $\beta = -81.9 \times 10^6$ cells/L) and Th17 cells ($P = 0.030$, $\beta = -2.5 \times 10^6$ cells/L) at day +90 in multivariate analysis, but were not associated with Tregs.
Fifteen patients (44.1%) developed aGVHD grade I-III at day +19. aGVHD was associated with increased numbers of Tregs ($P = 0.021$), decreased Th17/Treg ratio ($P = 0.0038$), and decreased Tc17 counts ($P = 0.042$) at day +90 in multivariate analyses, and with a trend towards less naïve T cell subsets.
Conclusion: This study suggests that IL-7 levels in the early post-HSCT phase play a differential regulatory role in the reconstitution of T cells, being associated with reduced numbers of pro-inflammatory Th17 cells and a decreased Th17/Treg ratio.
Prior aGVHD was associated with increased levels of Tregs at day +90, suggesting a regulatory response to increased alloreactivity related to aGvHD.
In conclusion, this study indicates that reconstitution of functionally different immunoregulatory subsets of T cells are differentially regulated during immune reconstitution post-HSCT, influenced by IL-7 levels and the degree of alloreactivity.
Disclosure of Interest: None declared.

P658

Reconstitution of lymphocyte and antigen-presenting cell (APC) subsets in peripheral blood (PB) following intrabone (IB) transplantation of allogeneic cord blood (CB) hematopoietic stem cells (HSC)

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Introduction: The IB injection of allogeneic CB HSC may allow to achieve better engraftment even with limiting amounts of total nucleated cells. However, to date the immune reconstitution of patients undergoing IB CB transplantation has not been thoroughly described.
Materials (or patients) and methods: we studied 21 adult patients enrolled in a single center phase II trial of allogeneic IB CB transplantation (NCT 00886522) at the University of Bologna between 2009 and 2012. The indications to transplant were AML ($n = 15$), ALL ($n = 5$) and MM ($n = 1$), mostly (20/21) in advanced phase. All patients received myeloablative pre-transplant conditioning; GVHD prophylaxis consisted of low dose (15-30 mg/kg) rabbit ATG-Fresenius (NEOVII), Cyclosporin A and Mycofenolate (MMF). Multicolor flow cytometry was employed to measure the numbers of circulating CD4+ and CD8+ T lymphocytes, B and NK cells, and the APC subtypes CD14+ and CD16+ monocytes, as well as conventional CD11c+ DC (cDC), CD123+ plasmacytoid DC (pDC) and CD16+ monocytoid DC in samples of PB harvested at one ($n = 11$), 3 ($n = 20$), 6 ($n = 15$) and 12 ($n = 8$) months after transplant. The data were compared to two historical (2001-2011) cohorts of patients receiving allogeneic PB stem cell (PBSC, $n = 155$) and bone marrow (BM, $n = 120$) transplants.
Results: the recovery of CD3+ and CD8+ cells at 1 through 6 months after transplant was significantly delayed as compared to both PBSC and BM transplants. However, the recovery of CD4+ T cells, albeit reduced after one month [5x10e6/l (IQ 3-6)], was comparable later on [131 (63-161) at 3, 162 (108-297) at 6 and 293 (87-497) at 12 months]. As previously reported following intravenous (IV) CB transplantation, the number of B cells increased to supranormal levels starting at 3 months after transplant [333x10e6/l (44-491) at 3, 678 (336-928) at 6 and 858 (143-1051) at 12 months]. Moreover, patients undergoing IB CB transplantation had increased numbers of pDC at 3 [7.1x10e6/l (2.2-13)] and 6 [9 (5-12)] months. Finally, the recovery of cDC, CD16+ DC and CD16+ and CD14+ monocytes, as well as of NK cells, was comparable in the 3 groups of patients.
Conclusion: patients undergoing IB CB transplantation have better reconstitution of B cells and pDC, and comparable reconstitution of CD4+ T cells as compared to patients receiving PBSC and BM grafts. Further studies directly comparing IB to IV CB transplantation are needed to evaluate whether the immune reconstitution is improved after IB CB transplantation.
Disclosure of Interest: None declared.

P659

Post-transplant monitoring of minimal residual disease by molecular marker identifies patients at high risk of relapse: a retrospective, single-center study of 144 patients with acute myeloid leukemia monitored by RT-PCR, flow cytometry and chimerism

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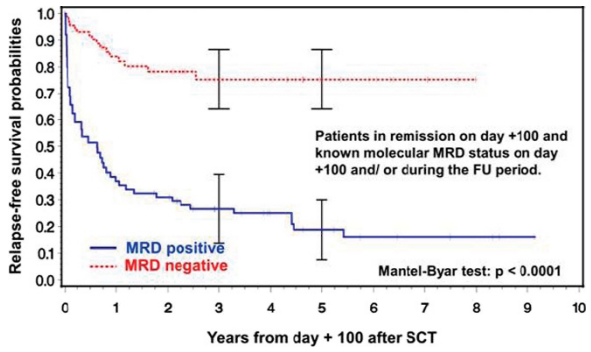
Introduction: The quantification of molecular markers, flow cytometry of leukemia-associated aberrant immunophenotypes (LAIP), and chimerism analyses are frequently used tools to monitor minimal residual disease (MRD) after allogeneic stem cell transplantation (SCT) in patients with acute myeloid (AML). However, their clinical implications are still limited and only little evidence of single molecular markers is available.
We report on a retrospective study of 144 patients with molecular MRD markers and analyzed the prognostic values of molecular MRD monitoring, baseline risk factors, and follow-up (FU) markers (e.g. chimerism analyses and LAIP).
Materials (or patients) and methods: Between January 2000 and August 2012, 495 AML patients underwent SCT at our institution. 164 patients had molecular MRD markers and 144 patients had at least one sample with quantitative results prior and/or after SCT, and were included into the analyses. At transplantation, 49 patients were in complete remission, while 95 patients were in a more advanced status of their disease (>CR2). Most patients received reduced intensity conditioning ($n = 142$). In 338 bone marrow samples MRD was monitored prior to SCT, on day +30 and on day +100. During the FU period after day +100, MRD was monitored at individual intervals in 429 peripheral blood samples. Quantitative RT-PCR was performed for NPM1 mutation ($n = 52$), MLL-PTD ($n = 31$), RUNX1-RUNX1T1 ($n = 12$), CBFß-MYH11 ($n = 14$), MLL rearrangements ($n = 20$), MDS-EVI1/EVI1 ($n = 10$), and DEK-CAN ($n = 5$). Sensitivities of the different RT-PCRs assays ranged between 10^{-4} and 10^{-6}.
Results: After a median FU of 41 months (range, 4–115), 43 patients (30%) relapsed. The MRD levels monitored by RT-PCR prior to SCT and on day +30 after SCT showed no significant impact on relapse-free survival (RFS) and overall survival (OS). At day +100 after SCT, MRD positivity was strongly associated with worse RFS (HR 3.1, $P = 0.001$) and OS (HR 3.2, $P = 0.004$). This was also reflected in a cumulative two-year incidence of relapse of 61% for MRD positive patients versus 15% for MRD negative patients ($P < 0.0001$). In addition, a change to MRD positivity during the FU period was strongly associated with a worse RFS (Mantel-Byar, $P < 0.0001$, Figure 1). Furthermore, in



univariate regression models we analyzed SCT baseline factors and FU markers. The remission status prior to SCT, the NPM1 mutation status, a HLA matched donor, the platelet count at day +100 (cut-off <50 G/l), and the European LeukemiaNet risk stratification (favorable versus others) were prognostic relevant risk factors. LAIP analyses (3-colors flow-cytometry, cut-off 0.1%) and a change in chimerism (STR-PCR and XY-FISH, cut-off 80% and 90%) of sorted (CD3+) and unsorted cells at the respective time points had no influence on RFS and OS.

Conclusion: Molecular MRD monitoring after SCT might be a useful tool to identify AML patients at high risk for relapse. In particular, MRD positivity on day +100 after SCT and the switch to MRD positivity during the FU period were significantly associated with worse RFS. Patients with MRD positivity on day +100 after SCT or with a switch to MRD positivity in the FU period may be considered for donor lymphocyte infusions (DLI) or chemotherapeutic interventions, such as hypomethylating agents.

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P660
Predictive value of cell-lineage chimerism analysis for relapse in Acute Myeloid Leukemia

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Introduction: Relapse is still a major obstacle to successful outcome in patients with Acute Myeloid Leukemia (AML). Minimal residual analysis for relapse prediction is complicated by high heterogeneity of molecular markers available in AML. Chimerism analysis, which can be used for almost all patients, has been shown to be useful for relapse prediction, especially when cell-lineage specific analysis is performed.

Materials (or patients) and methods: 183 AML patients were included in the study. Median age was 41 (range 1-69) years. Conditioning regimen was myeloablative in 111 patients and reduced in 72 patients. ATG was given to 128 patients. Stem cell source was PBSC ($n=141$), BM ($n=30$) and cord blood ($n=12$). Chimerism analysis was performed using the STR method in 74 patients and the more sensitive RQ-PCR method was used in 109 patients. For RQ-PCR, results showing >1% recipient cells were considered as mixed chimerism (MC). Blood samples, taken at regular time points after HSCT, were subjected to cell separation. For AML patients, CD33 was most commonly used as a myeloid marker.

Results: Follow-up time for patients still alive was 67 (14-186) months. Five-year relapse free survival was 55%. Patients showing donor chimerism or decreasing MC had low relapse/rejection risk and therefore grouped as Low-risk patients while patients with stable or increasing MC were grouped as High-risk patients. Five-year relapse incidence was 30% and 60% in the Low-risk and High-risk groups, respectively ($P=0.0001$). Due to chimerism results, donor lymphocyte infusion (DLI) was given to 29 out of 57 patients in the High-risk group. Relapse incidence in the DLI and the non-DLI groups was 53% and 67%, respectively ($P=0.047$). The time interval between MC detection and relapse was 4 (0-24) weeks. There was no significant difference between both chimerism methods, STR *vs* RQ-PCR. Also, lowering the threshold level for MC below 1% in RQ-PCR did not improve relapse prediction. The major reason for failure to predict relapse was isolated BM relapse where no increase of MC was detected in blood at all. For 5 patients in the High-risk group, long term MC was detected between 2 to 11 years without any signs of relapse.

Conclusion: As previously shown by others, there was a strong correlation between MC detection and relapse also in this study. Also, DLI was successfully used in some high-risk patients to prevent relapse. Since relapse was not predicted in patients with isolated BM relapse, regular BM sampling may be needed in AML.

Disclosure of Interest: None declared.

P661
Immune reconstitution after haploidentical transplantation in children with hematological malignancies: a retrospective analysis using CD3/CD19 or TCRαβ/CD19 depleted mobilized peripheral stem cell grafts

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Introduction: Immune reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT) is affected by many factors, being the cellular manipulation of the inoculums a major one.

Materials (or patients) and methods: We evaluated the kinetics of early immune reconstitution of children with hematological malignancies receiving an haploidentical transplantation using CD3/CD19 (group A) or TCRαβ/CD19 (group B) depleted mobilized peripheral stem cell graft. Sixteen patients (8 in group A and 8 in group B) were included in the study. All of them were in CR at time of transplantation and conditioned using the same regimen. Several leukocyte subpopulations were analyzed in peripheral blood at days +30 and +90 after transplantation using multiparametric flow cytometry.

Results: All patient engrafted. The median time to neutrophil and platelet engraftment were 13 days and 10 days respectively for the whole population. All patients had full donor chimerism at time of engraftment. At day +30 there were no differences on NK cell population between groups (430 A vs 600 B p; ns). We found that group B patients had higher numbers of circulating double-negative T lymphocytes (CD3+CD4-CD8-) (median 125/mL range; 50- 260 vs 4/mL range; 1-10 $P<0.006$); NKT (CD3+CD56+) (median 30/mL range; 16-160 vs 3/mL range; 0-17 p: 0.03) and terminally-differentiated (CD45RA+CCR7-) CD4 (median 4/mL range; 1-10 vs 1/mL range; 0-2, p: 0.04) and CD8 (median 8/mL, range; 4-14 vs 1/mL range; 0-4, p: 0.01) T lymphocytes at day +30 compared to group A. At day +90, the numbers of circulating T lymphocytes (CD45+CD3+) (median 910/mL range; 480-665 vs 225/mL range; 6-640) $P<0.05$) were significantly higher in group B than in group A. There were a higher cell numbers among the different subpopulations studied within the T cells,

TCRαβ (median 815/mL range; 250-2185 vs 80/mL range; 3-136, $P<0.05$) and TCRγδ (median 223/mL range; 47-460 vs 5/mL range; 1-13, $P<0.05$) for patients in group B. The same was true for B lymphocytes (CD45 + CD19 +)(median 430/mL range; 90-590 vs 50/mL range; 0-165, $P<0.01$), NKT lymphocytes, (median 59/mL range; 3-186 vs 2/mL range; 0-9, $P<0.03$) and dendritic cells (lineage-HLADR +)(median 56/mL range; 18-110 vs 16/mL range; 2-38, $P<0.02$). In addition, NK^{dim} ($CD3^{-}CD56^{dim}$) cell (median 330/mL range; 60-930 vs 56/mL range; 44-75, $P<0.05$) and type-1 (Lin-HLADR + CD11c +) dendritic cell (median 36/mL range; 8-110 vs 7/mL range; 0-27, $P<0.04$) numbers were also significantly higher in children transplanted with TCRαβ/CD19-depleted grafts. The number of infectious events after the first hospital discharge was lower among group B patients compared to group A (median 2 events, range; 0-3 vs 3 events, range; 0-12, $P<0.05$).

Conclusion: Our preliminary results suggest that the immune reconstitution during the first 3 months after transplant is faster and more robust when using a TCRαβ/CD19-depleted versus a CD3/CD19-depleted graft from a haploidentical donor in children with leukemia. Thymic output (circulating TCRαβ T lymphocytes) and T cell memory function (circulating CD45RA + CCR7- CD4 and CD8 T lymphocytes) seemed better preserved when using TCRαβ/CD19-depleted grafts.

Disclosure of Interest: None declared.

P662

Donor lymphocyte infusions in prevention of disease relapse in high-risk hematological malignancies after allo-HSCT with different donor types

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Introduction: Patients with a minimal residual disease (MRD) and/or a falling donor chimerism after allogeneic hematopoietic stem cell transplantation (allo-HSCT) are in high-risk group of disease relapse. Early usage of donor lymphocyte infusions (DLI) in this high-risk patient group may prevent disease relapse and extend disease free survival.

Materials (or patients) and methods: We retrospectively analyzed data of 63 patients with ALL ($n=32$), AML ($n=18$), CML ($n=7$), and MDS ($n=6$) who received DLI after allo-HSCT. The median pts age was 21 (range, 1–68) years. At the time of allo-HSCT only 19 (30%) pts had hematological, cytogenetic and molecular remission, 18 pts had minimal residual disease (MRD), and 26 pts were in active disease stage. Allo-HSCT were performed from matched related ($n=24$), unrelated ($n=33$; 10/10: $n=25$, 9/10: $n=8$), and haploidentical ($n=12$) donors, 6 pts received 2nd allo-HSCT from the same donor. Myeloablative (MAC) and reduced intensity conditioning (RIC) regimens were performed in 23 (33%) and 46 (67%) pts respectively. The source of stem cells was bone marrow (BM) in 31 (45%), and peripheral blood (PB) in 38 (55%) pts. Indications for DLI were MRD ($n=27$), falling donor chimerism ($n=18$), and high risk of disease relapse in pts who underwent allo-HSCT in active disease ($n=24$). Thirty (43%) pts received DLI in combination with additional therapy (hypomethylating agents, tyrosine kinase inhibitors, IFN-gamma, IL-2, GM-CSF), and 39 (57%) pts received DLI alone. Forty two pts received DLI by a bulk dose regimen (total cell dose (TCD) ranged from 1ч10^4 to 8ч10^7 CD3 +/kg), and 27 - an escalating dose regimen (TCD ranged from 2ч10^5 to 7.7ч10^8 CD3 +/kg).

Results: The median follow up was 10 months (mos) after allo-HSCT (range, 1-136 mos). CR after DLI was achieved in 15 pts (56%) with MRD. Falling chimerism was transformed in full donor in 7 (39%) pts after DLI. Duration of CR after DLI was 1-78 (median 6) mos. The relapse after DLI occurred in 29 pts (42%). RI was higher in pts after MAC vs RIC regimen, but it was not statistically significant (61% vs 38%, $P=0,1$). BM as stem cells source was associated with lower NRM (0% vs 35%, $P=0,002$), and higher RI after DLI (67% vs 32%, $P=0,02$) in comparison with PB. Acute graft versus host disease (GVHD) after DLI occurred in 15 (22%) pts, aGVHD grade III–IV - in 5 (7%) pts, extensive chronic GVHD - in 7 (10%) pts. The incidence of aGVHD was significantly lower in pts received DLI from full matched related or unrelated donors vs 1-mismatched or haploidentical donors (15% vs 42%, $P=0,02$). Administration of DLI after D + 100 was associated with lower aGVHD incidence than before D + 100 (13% vs 37%, $P=0,03$). Patients with aGVHD after DLI had significantly lower RI (18% vs 55%, $P=0,005$), but significantly higher NRM (52% vs 11%, $P=0,00024$) than ones without aGVHD. Causes of death ($n=27$) were: underlying disease ($n=15$, 24%), GVHD ($n=5$, 8%), infection ($n=5$, 8%), other ($n=2$). For all pts five-yr OS was 41,4%. At the time of follow-up 35 pts was alive, including 26 in CR.

Conclusion: Donor lymphocyte infusions is an effective method of disease relapse prevention in high risk patient group. RIC regimen, PB as stem cell source and aGVHD after DLI may be associated with lower RI. Acute GVHD incidence after DLI is acceptable.

Disclosure of Interest: None declared.

P663

Preemptive Immunotherapy based on Post-transplant Chimerism and MRD Monitoring is an Effective Strategy to Prevent Relapse after Allogeneic Stem Cell Transplantation in Children with ALL

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Introduction: Mixed chimerism (MC) and MRD strongly predict relapse in children with ALL after allo-SCT. Pre-emptive immunotherapy (IT), e.g. withdrawal of immunosuppression (WD-IS) or DLI can prevent impending relapse. In this study we retrospectively analysed chimerism and MRD monitoring and the effect of pre-emptive IT.

Materials (or patients) and methods: Patients: Between January 2005 and July 2014, a total of 89 pts with ALL (pB-ALL, $n=63$; T-ALL, $n=20$; biphen. ALL, $n=6$) received allo-SCT. 47 pts were in CR1, 26 pts in CR2, 15 pts were CR3 and 1 pt CR4 at time of transplant. Donors were MSD ($n=18$), MUD ($n=61$) and haploidentical ($n=10$). Conditioning consisted of TBI (12 Gy) and ETO in pts with a matched donor and FLU, THIO, MEL in haplo pts.

Methods: Chimerism was assessed weekly in peripheral blood until day 200 and monthly thereafter. Bone marrow analyses were done at days + 30, + 60, + 90, + 180 and + 365 post-transplant. Thereby, MRD was assessed in 56 pts, whereas no diagnostic material was available in 33 pts.

Results: 64/89 pts (72%) showed complete chimerism (CC) in all follow-up analyses, and 25/89 pts (28%) developed MC. From 56 pts in whom MRD could be assessed, 40 pts remained MRD negative and 16 developed MRD positivity after transplantation. IT (WD-IS, $n=12$; DLI, $n=14$) was initiated based on chimerism analysis in 22/25 pts with MC, and was guided by MRD detection in 4 pts. For the total cohort of pts, pEFS and pOS were 0.67 and 0.77, respectively. Cumulative incidence (CI) of TRM and relapse (CI-R) were 0.11 and 0.24 for all pts.

Chimerism: 8/64 pts (12%) who were always CC developed a bone marrow relapse. pEFS was 0.74 in CC-pts and 0.51 in MC-pts ($P<0.032$). 22/25 MC-pts (88%) received pre-emptive IT resulting in a pEFS of 0.58. Due to rapid progression IT was not initiated in 3/25 MC pts. All MC-pts without IT relapsed. While CI-TRM remained low (0.10 for CC resp. 0.12 for MC, $P>0.68$) in

both groups, CI-R was 0.17 in CC- and 0.41 in MC-pts ($P < 0.021$).

MRD: Pts were grouped according to their highest MRD value post-transplant in MRD negative, low positive (<10E-4), and high positive (⩾10E-4) pts. pEFS and pOS were 0.82 and 0.95 in MRD negative ($n = 40$), 0.56 and 0.88 in low level MRD ($n = 8$), and 0.25 and 0.25 in high level MRD ($n = 8$) positive pts ($P < 0.001$). CI-R was 0.14 in MRD negative, 0.36 in MRD low, and 0.75 in MRD high positive pts ($P < 0.001$), while CI-TRM between these groups remained low (0.05 for MRD negative, 0.13 for MRD low and 0.00 for MRD high, $P > 0.55$).

Multivariate analysis: Multivariate analysis for pEFS also indicated that MC and high level MRD were independent poor prognostic factors (MC, $P < 0.049$, RR 3.16 and high level MRD, $P < 0.022$, RR 3.99), while remission status before transplantation, ALL lineage, donor type, graft source, T cell depletion or sex showed no significant influence.

Conclusion: Our results show that analysis of chimerism and MRD allow the prediction of impending relapse in virtually all pts with ALL and that pre-emptive IT can improve outcome in these pts.

Disclosure of Interest: None declared.

P664

T-cell immune reconstitution predicts the outcome of Human Herpes Virus 6 Infection in 54 Patients after Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Human herpesvirus type 6 (HHV-6) is increasingly recognized as an opportunistic and potentially life-threatening pathogen in recipients of allogeneic hematopoietic stem cell transplantation (AlloSCT). Approximately 40% of patients after alloSCT experienced HHV-6 reactivation, increasing severe clinical complications and transplant-related mortality.

Materials (or patients) and methods: From January 2009 to February 2013, we retrospectively evaluated 54 consecutive adult patients (median age 50 years) who developed positivity to HHV-6 after alloSCT for high-risk hematological malignancies. Stem cell donors were family haploidentical (37), HLA identical sibling (8), unrelated volunteer (6), cord blood (3). The viral load was determined by quantitative PCR (Nanogen) in plasma, BAL, CSF, BM aspirates or in gastrointestinal biopsies.

Results: Median time from alloSCT to HHV-6 reactivation was 34 days (range: 0-705). Thirty-one patients presented HHV-6 positive in plasma, 9/54 in BM, 33/54 in gut biopsies or BAL, 7/54 in CSF. At the time of viral positivity all pts were receiving acyclovir as viral prophylaxis except five. Twenty-nine patients had acute graft versus host disease (GvHD). Twenty-two out of these twenty-nine patients experienced a grade III-IV acute GvHD, requiring high dose steroids in twenty-six cases. A concomitant CMV positivity was detected in 15/54 patients. The median absolute count of CD3+ lymphocytes was 207 cells/mcl. In 52/54 cases we reported HHV-6 clinical manifestations: fever (43), skin rash (22), hepatitis (19), diarrhoea (24), encephalitis (10), BM suppression (18), delayed engraftment (11). HHV-6 positivity led to antiviral pharmacological treatment in 37/54 cases, using as first choice therapy foscarnet. Amongst the total fifty-four patients with documented HHV-6 positivity thirty-one solved the clinical event. However the mortality rate was relatively high in this population (overall survival (OS) ± SE at 1 year after HHV-6 reactivation was 38% ± 7%), mainly related to infections or GvHD. A better OS is significantly associated with CD3+ cells ⩾200/mcl at the time of HHV-6 reactivation (OS at 1 year 63% compared to 11% for patients with CD3 <200/mcl; HR: 0.27, 95% CI 0.12-0.54, $P = 0.0002$). The overall survival of these patients was also positively affected by the absence of acute GvHD grade III-IV at time of viral reactivation (HR: 0.03, 95% CI 1.08-4.03, $P = 0.03$) and by the complete disease remission at time of HSCT (HR:0.26, 95% CI 0.07-0.89, $P = 0.03$). In this analysis the overall survival was not significantly influenced by steroids administration (HR: 1.36, 95% CI 0.71-2.60, $P = 0.36$), time after alloSCT (HR: 1.30, 95% CI 0.51-3.33, $P = 0.59$), type of antiviral prophylaxis (HR: 1.02, 95% CI 0.45-2.33, $P = 0.96$), plasma viral load (HR:1.18, 95% CI 0.51-2.76, $P = 0.69$) and organ involvement (HR:1.14, 95% CI 0.59-2.20, $P = 0.70$).

Conclusion: This retrospective analysis confirms a correlation of HHV-6 with high morbidity and mortality rates after alloSCT. Despite HHV-6 detection typically occurred early after alloSCT, a better T-cell immune reconstitution has the potential to clearly improve clinical outcome. These findings provide an intriguing window into the immune response to HHV-6, suggesting a more detailed analysis for lymphocyte subpopulations and for tracking viral cellular immune responses in this population.

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P665

Impact of CTLA4 genotype and other immune response gene polymorphisms on umbilical cord blood transplantation outcomes. A Eurocord, CBC-CTIWB, Netcord and FMRP-USP Study

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Introduction: Donor and recipient polymorphisms of immune defense and inflammatory cytokine genes have been associated with outcomes after bone marrow or peripheral blood stem cell transplantation (SCT), but have not been described in the setting of umbilical cord blood transplantation (UCBT).

Materials (or patients) and methods: In order to evaluate the impact of genetic polymorphism of recipients and UCB units on overall survival (OS) and other outcomes, we have selected the following candidate genes related to immune response: NACHT-leucine-rich repeat with a pyrin domain at N-terminus (NLRP1-rs5862, NLRP2-rs043684, NLRP3-rs10754558), Toll-

interleukin 1 receptor domain containing adaptor protein (TIRAP/Mal-rs8177374), interleukin-10 (IL10-rs1800872), V-rel reticuloendotheliosis viral oncogene homolog (REL-rs13031237), tumor necrosis factor receptor superfamily - member 1B (TNFRSF1B-rs1061622) and associated protein 4 of cytotoxic T lymphocyte (CTLA4-rs3087243). Seven Netcord banks provided DNA samples from 851 UCB units and 173 recipients. All patients underwent UCBT at EBMT centers.

Results: Among the 851 recipients, 57% were male, 61% adults. Degree of HLA matching between UCB unit and recipient (HLA -A, -B at antigen level and -DRB1 at allele level) was 6/6 in 12%, 5/6 in 40%, and $\leq$4/6 in 48% of transplants. Myeloablative conditioning was used in 77% of cases and anti-thymocyte globulin or monoclonal antibodies in 82% of cases. The median number of infused total nucleated cells (TNC) and CD34+ cells was $3.7x10^7$/kg and $1.6x10^5$/kg. About 80% of patients ($n = 696$) were transplanted for malignant diseases. In multivariable analysis of the overall cohort adjusted for patient-, donor- and disease-related variables, recipients of UCB units with GG CTLA4 genotype had a decreased OS (HR 1.36; 95%CI 1.03-1.80; $P = 0.03$), increased non-relapse mortality (NRM) (HR 1.41; 95%CI 1.24-1.59%; $P = 0.01$) and poorer neutrophil engraftment (HR 1.25; 95% CI 1.10-1.41; $P = 0.01$). In the cohort with malignancies, recipients of UCB units with GG CTLA4 genotype also had an increased NRM (HR 1.52; 95% CI 1.35-1.72%; $P < 0.01$) and inferior disease-free survival (DFS) (Figure 1; HR 1.41; 95% CI 1.06-1.88; $P = 0.02$), whereas the AA CTLA4 genotype was associated with lower relapse rate (HR 0.64; 95% CI 0.57-0.72; $P = 0.02$). Other gene polymorphisms were also associated with some UCBT outcomes. Recipients of TT IL-10 units showed better neutrophil (HR 0.77; 95% CI 0.68-0.87; $P = 0.03$) and platelet (HR 0.66; 95% CI 0.59-0.75; $P < 0.01$) recovery. Recipients of GG TNF CBU had lower platelet recovery (HR 2.15; 95% CI 1.90-2.43; $P < 0.01$) and of GG NLRP1 CBU had increased incidence of chronic GVHD (HR 1.51; 95% CI 1.33-1.71; $P = 0.03$). Recipient polymorphisms identified in the samples collected prior to UCBT ($n = 173$) were not associated with UCBT outcomes.

Conclusion: In this retrospective analysis, UCB unit gene polymorphisms in *CTLA4*, *IL-10*, *TNF*, and *NLRP*-1 influenced UCBT outcomes. Importantly, CTLA4 GG genotype of the graft was associated with inferior survival whereas an AA genotype predicted a lower relapse rate in patients with malignant disorders. These findings deserve further investigations and, if confirmed, the biological mechanisms involved in the association between CTLA4 polymorphism and UCBT outcomes would be elucidated.

Disclosure of Interest: None declared.

P666

Chimerism status after unrelated donor bone marrow transplantation with fludarabine-melphalan conditioning is affected by the melphalan dose and is predictive of relapse

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Introduction: Chimerism analysis after allogeneic hematopoietic stem cell transplantation (HSCT) enables us not only to detect the kinetics of engraftment but also to predict clinical events such as graft failure and disease relapse. However, there are few reports concerning the chimerism status after allogeneic BMT with reduced intensity conditioning (RIC) regimens. We prospectively analyzed lineage-specific chimerism and retrospectively evaluated clinical outcomes in patients who underwent unrelated donor BMT (UBMT) with fludarabine plus melphalan (FM) as the conditioning regimen. The purpose of this study was to investigate the predictive value of early kinetics of chimerism on primary graft failure, GVHD, overall mortality, non-relapse mortality (NRM), and relapse.

Materials (or patients) and methods: We studied 80 patients who underwent UBMT following FM conditioning at centers belonging to the Nagoya Blood and Marrow Transplantation Group from 2005 to 2012. Peripheral blood (PB) samples were obtained 14 and 28 days after UBMT. PB was separated into mononuclear cells (MNCs), erythrocytes and granulocytes. Furthermore, MNCs were separated into T-cells, NK-cells and other MNC fractions using immunomagnetic beads. DNA chimerism was analyzed using PCR of informative microsatellite regions. Complete donor chimerism (CDC) was defined as the presence of at least 95% donor DNA in all analyzed fractions, whereas mixed donor chimerism (MDC) was defined as the presence of more than 5% recipient DNA in at least one analyzed fraction. The potential association between the chimerism status and clinical events were analyzed by time-to-event comparisons.

Results: The median age was 55 years (range, 18-69). Diagnoses included AML ($n = 40$), ALL ($n = 7$), CML ($n = 5$), MDS ($n = 11$), malignant lymphoma ($n = 15$) and others ($n = 2$). GVHD prophylaxis was either a combination of tacrolimus and short-term MTX ($n = 73$) or cyclosporine and short-term MTX ($n = 7$). Moreover, HLA-A, B, C, and DRB1 alleles were matched in 42 donor-recipient pairs and mismatched at one, two, and three alleles in 23, 3, and 4 pairs, respectively. The median follow-up period for patients who were alive was 1053 days (range, 123-2574 days). The FM regimen comprised 125 mg/m^2 fludarabine and 90-180 mg/m^2 melphalan. Patients were divided into three groups according to the dose of melphalan: FM90-130 ($n = 9$), FM135-140 ($n = 22$), and FM150-180 ($n = 49$). On days 14 and 28, 43% and 10%, respectively, of assessable patients had MDC. Melphalan at $\leq$130 mg/m^2 was the only factor associated with an increased incidence of MDC at day 28 ($P = 0.03$, two-sided Fisher's exact test). In multivariate analysis, MDC at day 14 was associated with higher overall mortality (HR = 2.1; 95%CI, 1.1-4.2; $P = 0.04$) and relapse rate (HR = 3.0; 95%CI, 1.2-7.5; $P = 0.02$), but not with evidence of primary graft failure, acute GVHD, chronic GVHD or NRM.

Conclusion: The FM regimen yields prompt complete donor chimerism after UBMT, but melphalan at $\leq$130 mg/m^2 significantly decreased the level of donor chimerism. The chimerism status on day 14 may be a useful predictor of disease relapse and mortality after UBMT with FM regimens.

Disclosure of Interest: None declared.

P667

Immunosuppression Taper as the Sole Therapy for Early Relapse is Effective after Reduced Intensity Allogeneic Hematopoietic Cell Transplantation

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Introduction: For patients with early relapse after hematopoietic cell transplant (HCT) while still on immunosuppression (IS), IS taper alone may result in graft-versus-tumor (GVT) activity. We describe the course of 48 patients with disease recurrence that responded to IS taper without chemotherapy, radiation, or donor lymphocyte infusion.

Materials (or patients) and methods: We reviewed medical records of all patients with frank histologic or radiographic relapse ($n = 464$) and those with impending relapse defined by development of cytopenias with a fall in donor derived

chimerism ($n = 87$) within one year of HCT who were on IS at time of relapse between January 1, 2004 and December 31, 2013 at Dana Farber Cancer Institute. Complete response after IS taper was defined as complete recovery of blood counts, remission bone marrow biopsy, negative imaging, or chimerism recovery to nearly 100%. Survival probabilities were calculated using the Kaplan Meier method.

Results: 123 of 464 frank relapse patients were treated with IS taper alone, of which 34 patients responded. 33 of the 101 who had undergone reduced intensity conditioning (RIC) and 1 of the 22 who had undergone myeloablative conditioning (MAC) responded to IS taper alone at time of relapse (32.7% and 4.5% respectively; $P = 0.0073$). 14 of 87 patients with impending relapse responded to IS taper alone (16.1%). The median age of all patients responding to IS taper alone was 62 years at time of HCT. In the 48 patients who responded to IS taper alone, the diagnoses were MDS ($n = 14$), AML ($n = 11$), non-Hodgkin lymphoma ($n = 8$), CLL ($n = 6$), Hodgkin disease ($n = 4$), CML ($n = 3$) and multiple myeloma ($n = 2$). At time of HCT, 30 patients responding to IS taper alone had low/intermediate disease risk index (DRI) and 18 had high/very high DRI. 3 patients had active GVHD and 2 had resolved GVHD still on therapy prior to relapse.

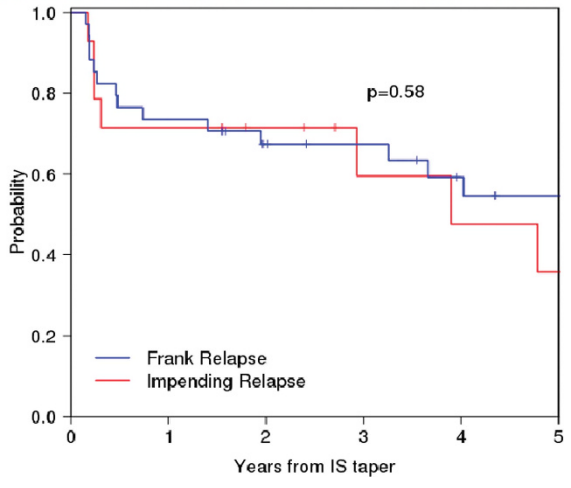
The median time to frank or impending relapse was 105 days (range 57-360) after HCT. The median interval between starting and completing IS taper was 30 days (range 0-251), but 13 patients could not come off IS completely due to development of GVHD. The median time to documented response after initiation of IS taper was 77 days (range 14-189). 35 patients had a complete response while 13 patients had a partial response. 9 patients subsequently relapsed late after initial response to IS taper at a median time of 2.38 years (range 0.88-3.95).

45 of the 48 patients developed or had a flare of previous GVHD as a consequence of IS taper (26 had grade II-IV aGVHD and 19 had cGVHD). The median time to developing GVHD after starting IS taper was 39 days (range 7-261). 25 patients died during follow-up (10 from GVHD, 6 from disease, 7 from infection, 1 from congestive heart failure and 1 unknown). The median follow-up time among survivors was 4 years (range 1.6, 9.4). The median overall survival (OS) time from IS taper was 4.78 years (95% CI 2.93-7.35). There was no difference in OS for frank versus impending relapse (figure 1).

Conclusion: IS taper alone in patients who relapse early after HCT can produce durable remissions but is almost always associated with development of GVHD. This benefit is almost exclusively observed in RIC HCT. When feasible, this strategy should be given sufficient time to allow for a GVT response to develop in order to avoid the adverse effects of more aggressive therapies.

Disclosure of Interest: None declared.

Figure 1. OS of Frank versus Impending Relapse in Patients Undergoing IS Taper Alone



P668

Monitoring of bone marrow chimerism after allogeneic HSCT—Early relapse detection by a quantitative PCR approach

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) has become a cornerstone in the treatment of patients at high risk for relapse of their acute leukemia.

Monitoring of chimerism has been designated to confirm engraftment, but, since the malignant clone is host derived, the increase of host chimerism might also allow prediction of relapse. However, commonly used STR assays show limited sensitivity, whereas XY-FISH, CD34+ cell chimerism and MRD approaches are limited by their applicability.

Materials (or patients) and methods: In this prospective, non-interventional study we evaluated the accuracy, reliability and feasibility of a quantitative PCR based commercially available assay (AlleleSEQR® Chimerism Assay, Abbott) and its potential for early relapse detection.

From 05/11 to 01/13 a total of 95 consecutive patients, receiving allo-HSCT for AML/MDS, were enrolled.

Results: Screening revealed traceable, host specific markers in all evaluable patients ($n = 68$). Even more than one discriminating marker was found in >89% of all related and in all unrelated donor/ host pairs. The in vitro detection limit, using 100ng artificially spiked DNA/well, was proven to be <0,05%. The overall time of testing was less than four hours.

According to local standards, bone marrow was assessed repeatedly after allo- HSCT, including day +30, +90 and +180. Those samples were used for host quantification. In order to determine the value for relapse prediction, at least two consecutive, leukemia free (CR) samples were required. Hence, 61patients were eligible for clinical correlation and were followed until 03/14.

Assessing the course of host chimerism, as quantified by PCR (qPCR), allowed convincingly reliable relapse prediction (86% vs. 15%, $P = <0,001$, Mantel-Byar-test). The median time from positive testing to relapse was 68 days (25-201), however, most remarkably, there was no relapse within >60days after negative testing, translating into an estimated specificity of ≥97% for a 120d period after testing.

Conclusion: In conclusion, we demonstrated the qPCR approach as a fast and highly sensitive tool for chimerism monitoring. The reliable and dynamic classification of the patient's risk for imminent relapse facilitates personalized, chimerism-triggered therapy. In perspective we will validate this assay for peripheral blood samples, allowing more frequent and convenient testing.

Disclosure of Interest: R. Reibke Funding from: study was supported by Abbott GmbH&CoKg, A. Dick: None declared, E. Hoster: None declared, M. Hubmann: None declared, R. Henschler: None declared, W. Hiddemann: None declared, K. Spiekermann: None declared, J. Tischer: None declared.

P669

Long-term follow-up of autologous stem cell transplantation in acute lymphoblastic leukemia. A single center experience

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Introduction: The possibility of studying minimal residual disease (MRD) at the immunophenotypic and\or molecular level in patients with acute lymphoblastic leukemia (ALL) has recently renewed the interest on the use of high-dose therapy

followed by autologous stem cell reinfusion (AuSCT) in selected cases with high-risk ALL ineligible for allogeneic transplantation. The aim of our study was to retrospectively analyze the long-term outcome of a consecutive cohort of ALL patients who underwent an AuSCT at our institution between 1996 and 2014.

Materials (or patients) and methods: This series includes 24 patients, 13 males and 11 females, with a median age at transplant of 31 years (range: 10-68). Five patients were transplanted in 2nd CR and 19 in 1st CR, because they were considered at high risk. In particular, 3 cases were Ph+, 3 carried a t(4;11), 2 were prednisone poor responders and 9 had a high white blood cells count (WBC) at the onset of the disease, 5 of which having a T-cell lineage affiliation. The stem cell source was peripheral blood in 22 patients and bone marrow in 2. In all cases, it was possible to assess MRD levels in the bone marrow and in the apheretic product prior to AuSCT, by means of immunophenotypic or molecular assays. At the time of transplantation, 5 patients were MRD positive and 19 negative in the bone marrow. In one case only, MRD evaluation on the apheretic product proved positive.

Results: Currently, 11 patients are alive in complete remission after a median follow-up of 73 months after transplant; 13 have relapsed after a median of 16 months (range 2-62). The 5-year projected probability of OS and DFS of the whole population is 64% and 45%, respectively, with a median DFS of 61 months. The only variable having a significant impact on DFS in univariate analysis is MRD negativity in the bone marrow at the time of transplant: 5-year DFS: 66% *vs* 0% for MRD negative *vs* MRD positive patients; the median DFS for patients MRD- has not been reached (*P*: <0.0000).

Conclusion: Although the sample size is relatively small, our data confirm the value of MRD detection and monitoring in ALL patients, also in the AuSCT setting. A prospective evaluation of its role in the therapeutic strategy of high-risk ALL within controlled clinical trials is warranted.

Disclosure of Interest: None declared.

P670

Recovery of Mucosal Associated Invariant T cells after allogeneic hematopoietic stem cell transplantation in children

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Introduction: Mucosa-associated invariant T (MAIT) cells are innate-like T cells expressing a semi-invariant T cell receptor (Vα7.2-Ja33 TCRa chain) that recognizes microbial-derived riboflavin precursor derivatives. At birth, MAIT cell levels are very low (0.1-1% of T cells in cord blood) and express naïve phenotype characteristics. They progressively expand during the first years of life in the presence of the commensal microbiota, and reach 1-10% of peripheral blood T cells with mature phenotype characteristics in adult blood. Given their mucosal localization, their capacity to secrete IL-17, and their anti-microbial functions, MAIT cells may be important after allogeneic hematopoietic stem cell transplantation (HSCT). However, their recovery after HSCT remains to be defined.

Materials (or patients) and methods: We prospectively analyzed MAIT cell reconstitution in 44 children who underwent HSCT from matched related donor (MRD, $n=32$) or umbilical cord blood (UCB, $n=12$) during the last 2 years. MAIT cell frequency and absolute numbers were determined in the donor and in the recipient before conditioning and at sequential intervals after grafting using 10-colour flow cytometry, in parallel to iNKT, NK, gdT, conventional T and B cells. In parallel, we compared the phenotypic and functional characteristics of cord blood and adult peripheral blood MAIT cells.

Results: In MRD recipients, MAIT cells rapidly recovered, reaching frequency similar to that in the corresponding donor as soon as 1 month after grafting (mean 3.5% of T cells). Moreover, they retained the mature phenotype of donor MAIT cells (>50% CD45RO+, 80% CD8aa+), suggesting that they represented donor-derived cells transferred with the graft. Surprisingly, around 6 months after grafting, MAIT cell numbers and percentages significantly dropped, and no production of new naïve MAIT cells appeared thereafter. In UCB recipients, MAIT cell frequencies and absolute numbers remained extremely low and not significantly different from those in cord blood. MAIT cells retained a naïve phenotype (>90% CD45RA+, 70% CD8ab+) until 3 months after grafting, and progressively acquired memory markers thereafter. *In vitro* experiments showed that, in addition to differences with adult blood cells in terms of cytokine/chemokine receptor expression and effector functions (cytokine production, cytolytic ability), cord blood MAIT cells had a greater proliferative capacity in response to mitogens.

Conclusion: In MRD recipients, the early MAIT cell reconstitution likely results from homeostatic proliferation of donor-derived cells transferred with the graft. However, this recovery is temporary and not relayed by thymic-dependent production of new naïve MAIT cells, at least during the first year after HSCT. In UCB recipients, a profound and prolonged MAIT cell lymphopenia is observed, although MAIT cells exhibit a strong intrinsic proliferative capacity in vitro. Whether early thymic T-cell progenitors that develop in the thymus after HSCT may give rise to new MAIT cells remains an open issue. Alternatively, it may be that newly produced MAIT cells initially migrate to mucosal tissues, and will only become detectable in the periphery after several months or years.

Disclosure of Interest: None declared.

P671

Anti-Torque teno virus immunity in patients undergoing allogeneic hematopoietic stem cell transplantation

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Introduction: Patients (pts) undergoing allogeneic hematopoietic stem cell transplantation (HSCT) are at high risk of infections. Slumbering infections resulting from previous anti-neoplastic treatments may be a contributing factor. Whether immunity at the day of HSCT differs between individual pts is not known.Torque teno virus (TTV) is a small non-enveloped, single stranded DNA *Anellovirus* with prevalence in the population of more than 90%. Infections are latent in immunocompetent individuals but viral loads increase greatly in pts with immunodeficiency. Because TTV is not sensitive to antiviral therapy, viral copies in plasma may be an appropriate parameter to measure immunocompetence in pts undergoing HSCT.

Materials (or patients) and methods: We used TaqMan©-based quantitative PCR to measure TTV titers in 104 adult pts receiving a first allogeneic HSCT for hematological malignancies. Patient groups were compared using non-parametric Mann Whitney test.

Results: At transplant (d0 ± 2 weeks), 28 pts (27%) had high numbers [≥90th percentile of 74 Controls (median $0.17\text{x}10^3$, interquartile range (IQR) $0.025\text{-}1.7\text{x}10^3$)] of viral copies in their blood while TTV titers in the others were normal ($TTV^{low/-}$). The latter was most likely evidence of sufficient residual immunity rather than of absence of virus because the number of viral copies in the 58 $TTV^{low/-}$ pts followed during the first months post-transplant, rose sharply between 1 and 2 months, a lag-time very similar to the one observed after the start of immunosuppression in organ transplantation. We found no significant impact of the number of chemotherapy cycles received or of time between HSCT and time of diagnosis or of last treatment.

TTV-titers at transplant were strongly associated with the type of disease. Viral copies in the 12 ALL and 9 NHL pts [$67\text{x}10^3$

(IQR $0.7x10^3$-$1.57x10^6$) and $218x10^3$ (IQR $15.4x10^3$-$10.3x10^6$) respectively] were significantly higher ($P<0.0017$;$P<0.001$) than titers in the 56 AML pts [$0.14x10^3$ (IQR $0.025x10^3$-$3.32x10^3$)] or titers in the 27 pts with other malignancies [$0.16x10^3$ (IQR $0.025x10^3$-$1.16x10^3$)] ($P<0.0017$;$P<0.001$). Titers in the 5 Ph^+ ALL pts [$2.0x10^6$ (IQR $112.4x10^3$-$38.5x10^6$)] were significantly higher ($P<0.018$) than in their 7 Ph^- counterparts [$1.0x10^3$ (IQR $0.1x10^3$-$113.8x10^3$)].
Most ALL/NHL pts had received long-term prednisone and NHL pts had received autologous transplantation while Ph^+ ALL pts had been treated with tyrosine kinase inhibitors. However, none of the non ALL/NHL pts that had received autologous transplantation (15), long-term-prednisone (11) or tyrosine kinase inhibitors (8) had high TTV-titers in their blood.
Conclusion: TTV titers increase greatly in pts after HSCT and may therefore be a candidate test to quantify immunity. Titers at HSCT are associated with the type of disease rather than with previously received therapies. High titers associated with NHL and Ph^+ ALL suggest that lymphocyte malignancies with large, destructive proliferations may have a more profound impact on immunity than other hematological malignancies.
Disclosure of Interest: None declared.

P672
Abstract Withdrawn

P673
The abnormal increased CD3-CD16 + CD56- NK subset may be the major effective cells which exert graft-versus-leukemia effect after the umbilical cord blood transplant

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Introduction: Cord blood transplantation (CBT) is being increasingly used for treatment of hematological malignancies.Our clinical analysis revealed the outcome of CBT for high risk acute leukemia relapse after HLA-mismatched CBT was lower than that BMT or PBSCT. It suggest that immunocompetent cells other than T cells may mediate the GVL effect. Because the recovery of NK cells promptly after CBT, so it is probably that NK cells may be more likely to contribute to the development of GVL effect.We found the $CD3^-CD16^+CD56^-$NK cell subset increase abnormally suggesting that this subset may be associated with the GVL after CBT. In this paper our purpose is to explore the characteristics of NK cells' reconstruction after CBT by detecting NK cells and their functional phenotype on their subsets. We intend to research CD3-CD16 + CD6-NK subset by comparison with CD3-CD56dimCD16 + NK subset.
Materials (or patients) and methods: We selected 121CBT and 60 BMT patients who were treated by myeloablative conditioning regimen, another 21 healthy donors as control. We detected the expression of CD11b, CD27 and CD57 on NK and their subsets by FACS at different time after transplant. We randomly selected 14 patients who are accepted CBT after 4 months and detect the activating and inhibitory receptors, granzyme/perforin and TNF/IFN-γsecretion.Mann-Whitney T test or paired T-test was used to determine whether there was a statistically significant difference.
Results: The frequency of NK cells in lymphocytes in receptors of CBT was less than BMT group from 3 month to 2 years, but the absolute numbers was below only within 1 month. Both the proportion and the absolute member of $CD3^-CD56^-CD16^+$ NK in CBT group were higher than that of BMT group after 4 month. The phenotype and function related to $CD3^-CD56^-CD16^+$NK subsets showed: The expression of CD11b or CD27 in CBT group was similar to BMT group, but the expression of CD57 was lower than that of BMT group or control from 2 to 9 months ($P<0.01$). Compared with $CD3^-CD56^{dim}CD16^+$ NK subset:The expression of CD11b on $CD3^-CD56^-CD16^+$ NK subset was no significantly different; the expression of CD27 reduced in 2 years and the expression of CD57 reduced in 3 month. However, the patterns of expression were different completely in healthy control. The expression of CD11b or CD57 was lower;CD27 was higher obviously in healthy control. Compared with $CD3^-CD56^{dim}CD16^+$ NK subsets: The expression of activating receptors of NKG2D,NKp46 and NKp30 reduced significantly on $CD3^-CD56^-CD16^+$ NK subsets; Inhibitory receptors of CD94 and CD158a also significantly reduced.; The expression of CD107a or intracellular IFN-γ was similar; The expression of intracellular reduced significantly ($P<0.01$).
Conclusion: Although the proportion of NK cells in lymphocytes in receptors of CBT was less than BMT group within 2 years, but the absolute numbers of NK was not reduced. The phenotype of immune reconstitute NK cells was naive after CBT, but the function was not reduced. $CD3^-CD16^+CD56^-$NK subset increased significantly, they ware completely different from the naive phenotype of this subset in healthy controls. The function and differentiation of this NK subset were in the state of mature. The function of this NK subset was similar to the cytotoxicity of $CD3^-CD56^{dim}CD16^+$ NK cells and this subset may be associated with increased GVL function after CBT.
Disclosure of Interest: None declared.

P674
impact of the different approaches of in vivo T cell depletion on NK cell subsets reconstitution after allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: T cell depletion (TCD) represents a well established strategy for graft-versus-host disease (GVHD) prevention following allogeneic hematopoietic stem cell transplantation (HSCT).Unfortunately,TCD is associated with delayed T-cell immune-reconstitution responsible of higher rates of disease recurrence and infection. Several approaches of TCD exist,including *in vitro* graft manipulation and *in vivo* administration of lympho-depleting agents. While all TCD approaches significantly affect T-cell reconstitution after HSCT,little is known about their effect on NK cells,major players in anti-tumoral and anti-infectious immunity after HSCT.In this study we assessed the impact of two different TCD approaches employed at our institution,*in vitro* alemtuzumab graft treatment and *in vivo* polyclonal antithymoglobulin (ATG) administration, on NK cell reconstitution after allogeneic HSCT.
Materials (or patients) and methods: We retrospectively analysed 714 blood samples from 123 patients transplanted at our center between 1995 and 2014, for which T and NK cell numbers were available on at least three occasions during the first year after HSCT.Twenty five patients received partial TCD (pTCD) grafts, consisting of *in vitro* alemtuzumab incubation before infusion followed on day + 1 by add-back donor TCD3 + cells.Thirty-seven patients received *in vivo* ATG administration, consisting of either Thymoglobuline (Genzyme) or ATG-F (Fresenius). Forty-four patients had combined ATG and pTCD.Seventeen patients had no TCD.Linear regression analysis was employed to compare T-cell and NK-cell reconstitution kinetics in patients receiving T-cell replete grafts with patients treated with different TCD protocols.A confirmatory cross-sectional analysis was performed at 1, 6 and 12 months using Mann Whitney test.
Results: As expected,T-cell reconstitution was significantly delayed in patients receiving pTCD grafts ($P<0.0001$), *in vivo* ATG ($P<0.0001$) or both ($P<0.0001$) compared to recipients of non TCD grafts.Importantly,no significant difference was observed in T-cell reconstitution kinetics among

groups of TCD patients treated with different methods. We found similar NK cell counts at 1 month after HSCT in recipients of T-cell replete grafts compared to patients receiving pTCD, ATG or combined pTCD/ATG.During the first year after HSCT,NK-cell reconstitution was not affected by either pTCD or ATG alone,while we observed a significant expansion of NK cells in recipients of combined pTCD and ATG when compared with not T-cell depleted group ($P = 0.0003$). This observation was confirmed in classical cross-sectional analysis performed at 12 months after HSCT [pTCD/ATG 180 cells/μl (IQR 120-370), no TCD 100 cells/μl (IQR 0-130); $P = 0.0366$].When NK subsets heterogeneity was taken into account,we observed a similar decline in $CD56^{bright}$ NK cells in all groups studied while we found a preferential expansion of the $CD56^{dim}$ NK cell compartment in combined pTCD/ATG treated patients compared with recipients of T-cell replete grafts.

Conclusion: NK reconstitution was not affected by either pTCD or ATG alone, while combined pTCD and ATG was associated with rapid and sustained NK cell expansion in the first year after allogeneic HSCT. These data extend our knowledge about the effects of TCD on immune-reconstitution after HSCT. Further studies are needed to evaluate the clinical impact of such results.

Disclosure of Interest: None declared.

P675

Abstract Withdrawn

Paediatric issues II

P676

Lung function and T cell reconstitution following allogeneic haematopoietic stem cell transplantation in children

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Introduction: Long-term survivors of paediatric HSCT are susceptible to complications due to chemotherapy, infections and Graft-versus-Host Disease (GvHD). In the lungs, chronic GvHD causes progressive obliteration of peripheral airways, but the pathogenesis is not fully understood. The current study aimed to investigate the correlation between changes in pulmonary function and recovery of lymphocyte subsets post HSCT.

Materials (or patients) and methods: We performed a population based, prospective, longitudinal study on 27 paediatric patients (4.2-15.8 years) who underwent HSCT in Denmark from 2010-2013. Seventeen patients (63%) had a malignant diagnosis. Donors were either siblings ($n = 4$), matched unrelated donor ($n = 17$) (bone marrow graft), or unrelated cord blood grafts ($n = 2$). Pulmonary function (forced expiratory volume in 1 second (FEV_1) and diffusion capacity of the lungs for carbon monoxide corrected for alveolar volume (D_{LCO}/V_A)) was measured before HSCT and subsequently 7 times until day +360. Patients were screened for cGvHD according to National Institute of Healths guidelines. T cell subsets were counted by flow cytometry at day +90 and +180 and defined as follows; CD4+ T cells, CD8+ T cells, CD4+CD161+CD196+ Th17 cells, CD8+CD161+CD196+ Tc17 cells and $CD4+CD25^{high}CD127^{low}$ Tregs. Correlations between pulmonary function values at day +90, +180 and +360, Th17, Tregs, Th17/Tregs-ratio at day +90 and +180 and the occurrence of cGvHD were tested with non-parametric statistics.

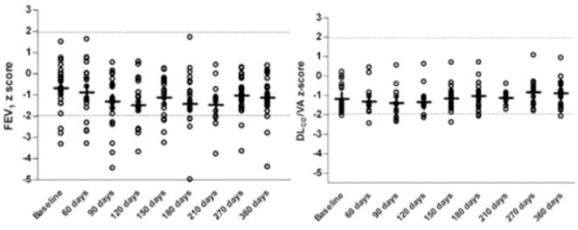

Figure: Pulmonary function development in time from HSCT in 27 Danish children. The horizontal lines represent mean values. The dotted lines represent limits of normality (+/- 1.96 z-score). Abbreviations:FEV1 forced expiratory volume in 1. second, DLCO/VA diffusion capacity of the lungs for carbon monoxide, adjusted for volume of air.

Results: Lung function declined following HSCT, most pronounced during the first 4 months (Figure 1). We found a significant positive correlation between Tregs at day +180 and mean FEV_1 z-score at day +360 (Spearman rho 0.662, $P = 0.010$) but neither Th17 nor Th17/Treg-ratio were associated with mean FEV1 at any time point. We found no significant correlation between the Th17 or Treg cell subsets and mean D_{LCO}/V_A z-scores at any time point during the first year. There was a borderline significant association between the occurrence of cGvHD and the value of FEV_1 at day +90 (median z-scores -2,84 vs. -0.87, $P = 0.076$) and day +180 (-2,50 vs. -1,15, $P = 0.069$). D_{LCO}/V_A values were not associated with cGvHD.

Conclusion: This is the first study to correlate pulmonary function with recovery of specific T-lymphocyte subsets post HSCT. In line with retrospective studies from our group, we found that the most severe decrease in pulmonary function took place during the first 4 months after HSCT. The significant correlation between Tregs day +180 and increased mean FEV_1 z-score at day +360 could possibly be explained by the anti-inflammatory effect of Tregs. Future studies including more detailed analysis of inflammatory parameters, microbiology and dynamics of lymphocyte recovery post HSCT (including NK- and B-cells) may contribute to a better understanding of the pathogenesis of the pulmonary complications following HSCT.

Disclosure of Interest: None declared.

P677

Efficacy of JAK1/2 inhibitor ruxolitinib in severe GVHD with capillary leak syndrome in a 12 years old boy

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Introduction: GVHD (graft-versus-host disease) remains a major complication of allogenic hematopoietic stem cell transplantation (HSCT). New therapeutic approaches recently targeted the response to the overload production of cytokines that characterizes GVHD. Ruxolitinib, a Janus kinases (JAK) inhibitor recently showed promising results in steroid-refractory GVHD in 6 adult patients. We report a 12 years old boy treated with ruxolitinib for active cutaneous GVHD and severe capillary leak syndrome.

Materials (or patients) and methods: A 12 years old boy suffering from combined immune deficiency was transplanted

with a 10/10 matched unrelated donor. He was conditioned by campath, busulfan and fludarabine. GVHD prophylaxis consisted in ciclosporin (from D-1) and mycophenolatemofetil (MMF) (from D0). Engraftment took place on D34 after transplant. Life threatening severe systemic adenovirus (ADV) infection occurred two months post-HSCT associated with poor hematologic and immunologic reconstitution. This condition required a non-manipulated stem cell boost 3 months post-HSCT allowing rapid and long lasting control of ADV infection and improvement of hematologic recovery. 32 days after the boost, he presented cutaneous (grade III), hepatic (grade II) and digestive (grade IV) acute GVHD associated with thrombotic microangiopathy (TMA) and chronic capillary leak syndrome. These conditions required several lines of treatments (ciclosporin, sirolimus, MMF, anti thymocytes globulin, anti-TNFa therapy, anti-IL2 therapy and extracorporeal photopheresis (ECP)) allowing partial responses. Eculizimab was also proposed in treatment of TMA and capillary leak syndrome. One year post-HSCT, acute skin GVHD grade III associated with life threatening severe capillary leak syndrome associated with cortico-dependence at 1 mg/kg persisted despite MMF and ECP treatment.

Results: JAK1/2 inhibitor, ruxolitinib, was introduced, proposed at the dose of 5 mg twice daily. In two weeks, impressive clinical improvement was noticed with loss of oedema (allowing a total weight loss of 8 kilos) and complete remission of skin GVHD. After 3 months of ruxolitinib, the patient is still in complete remission of skin GVHD and capillary leak syndrome without side effects of ruxolitinib. Steroids could be taped to 0.2 mg/kg per day and other immunosuppressive therapeutics could be stopped (MMF, ECP).

Conclusion: We report the first paediatric patient treated with ruxolitinib for persistent acute skin GVHD and capillary leak syndrome. This treatment was associated with a rapid and remarkable efficacy of both conditions but the follow up is still short. These encouraging results need to be confirmed in a prospective study. Infectious risk related to ruxolitinib has to be considered in these high-risk patients.

References: Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. Spoerl *et al.*, Blood 2014 Jun 12;123(24):3832-42

Disclosure of Interest: None declared.

P678

Haploidentical HSCT with Post-Transplant High-Dose Cyclophosphamide in Children and Adolescents with High Risk Hematological Malignancies. A Retrospective Multicenter Study from the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)

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Introduction: The use of post-transplant high dose cyclophosphamide is a innovative procedure to perform unmanipulated haploidentical stem cell transplantation (Haplo-PTCY).

Materials (or patients) and methods: From January 2012 to November 2014 29 pediatric patients with malignancies received an Haplo-PTCY (13 ALL, 6 AML, 1 dendritic cell leukemia, 1 CML, 2 NHL, 2 HD, 4 MDS) in 5 AIEOP-BMT centers. Seventeen patients were male (58%) and 12 females (40%). Their median age at HSCT was 8.9 years (1-21). Donor was the mother for 17 pts (57%), the father for 9 pts (30%), a brother for 2 pts (6%) and finally a sister for 2 pts (6%). Fifteen patients received a standard nonmyeloablative conditioning (Luznik L et al, BBMT 2008; 14: 641-50) including Fluradarabine 150 mg/m^2 and Cyclophosphamide 29 mg/Kg and total body irradiation 200 cGy, while the others received a Busulfan based or TBI 1200 cGy based conditioning regimen. Seven patients out of 29 were in CR1 (24%), 8 patients were in CR2 (27%), 13 patients had more advanced disease (45%). Four patients failed a previous allogeneic HSCT (14%). GVHD prophylaxis included Cyclophosphamide 50 mg/kg on day +3 and +4 and tacrolimus or cyclosporin-A plus mofetil mycofenoplate.

Results: Data were analyzed as of December 1st 2014. Median time to ANC and platelet engraftment was 17 days (14-26) and 27 days (16-71), respectively. At 1-year from Haplo-PTCY OS for the entire population was 74% (95% CI 58-91), RI cumulative incidence was 30% (95% CI 17-52), TRM was 10% (95% CI 17-52), acute GVHD II-IV was 20% (95% CI 10-41), acute GVHD III-IV was 3% (95% CI 0-23) and chronic GVHD was 4% (95% CI 0-40). Among TRM causes: one patient died early after Haplo-PTCY following blinatunomab courses given to obtain leukemia remission of a post-HSCT-relapsed refractory ALL, one patient died of septic shock and one for pneumonia. OS according to conditioning regimen was 85% (95% CI 66-100) and 59% (95% CI 31-87, $P = 0.19$) for standard nonmyeloablative conditioning compared to other Busulfan or TBI 1200 cGy based combination. OS was 81% when we donor was the mother (95% CI 62-100) while it was 64% (95% CI 32-97, $P = 0.34$) when the father was chosen as donor. Considering NK alloreactivity OS was 81% (95% CI 57-100) compared to 60% (95% CI 33-88) when alloreactivity was not present ($P = 0.21$). Among the 4 patients having a previous alloHSCT 2 patients relapsed at 38 and 143 days, one patient died of pneumonia at 49 days and finally one patient is alive and leukemia-free at 744 days post Haplo-PTCY. At day +60, 83% of the patients achieved full-donor chimerism, one patient had stable mixed chimerism (25% donor) with no disease recurrence, one patient had mixed chimerism (donor 60%) followed by leukemia relapse, one patient relapsed before day +60. For 2 patients we have no data available on day +60 about donor-recipient chimerism. At day +60 median CD4+ cells/μL were 117 (32-1159), CD8+ cells/μL were 374 (48-4909), CD19+ cells/μL were 46 (0-600) and finally CD56+16+ cells/μL were 150 (12-365).

Conclusion: In conclusion children and adolescents may benefit from a Haplo-PTCY when a related or unrelated compatible donor is unavailable. A comparative study between Haplo-PTCY and other alternative donor HSCT is needed.

Disclosure of Interest: None declared.

P679

Etanercept for the Treatment of Steroid-Refractory Acute Graft-Versus-Host Disease: a single centre experience in children

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Introduction: Steroid-refractory acute graft-versus-host disease (aGVHD) remains a clinical challenge, for which standard therapy has not yet been defined. Monoclonal antibodies against tumor necrosis factor-a (TNF-a) have been proposed for their potential activity in steroid-refractory aGvHD.

Materials (or patients) and methods: From January 2009 until November 2014,16 children were treated with human

tumour necrosis factor receptor p75 Fc fusion protein (etanercept) because of refractory acute GVHD (r-aGVHD) (13/16 grade 3-4) occurring after allogeneic haematopoietic stem cell transplantation (HSCT) performed at G. Gaslini Institute.
Organs involved were skin in 13 cases (6 grade 3-4), gut in 13 (10 grade 3-4) and liver in 4 (all grade 1).
Etanercept was administered at the dose of 0.4 mg/kg (to maximum 25 mg) s.c. twice weekly for a total of 16 doses (8 weeks). Overall response (OR) and single-organ response have been evaluated after 4 doses, 8 doses and at the end of treatment (16 doses).
Results: Fourteen children completed the schedule of 16 doses, 2 patients with active r-aGvHD died before the end of treatment for severe infectious events after 4 and 8 doses respectively.
At the intermediate evaluations after 4 and 8 doses, among evaluable patients the OR was 62,5% (complete response [CR] in 31,25%) and 80% (CR 46,6%) respectively. In particular OR by organ after 4 and 8 dose was 69,2% (CR 53,8%) and 80% (CR 73,3) for skin, 46,1% (CR 23%) and 61,5% (CR 46,1%) for gut.
OR at the end of treatment evaluated on 14 children was 93% (CR in 71,4%), OR by organ was 91% for skin (all CR) and 100% for gut (CR 63,6%). Response of hepatic GvHD wasn't considered because of the low number of patients and absence of high grade.
Among high grade of r-aGvHD (grades 3-4), the OR at the end of the treatment was 71.4%(CR 50%).
Three life-threatening infectious episodes (1 sepsis by candida, 1 severe central nervous system zygomicosis, and 1 sepsis by serratia marcescens) were recorded after the beginning of etanercept therapy.
Overall, 62,5% of patients were alive at a median of 1 year after HSCT; 30% of them developed chronic GVHD. Six patients (38%) died:active r-aGvHD was the main cause of death in 4, leukemia recurrence in 2.
Conclusion: The high rate of response observed in our experience, suggested that etanercept may be a useful and safe treatment for refractory and steroid-resistant/dependent aGVHD, specially in patients with intestinal involvement. In particular, most of responses were obtained after the first 8 doses for cutaneous and lately, at the end of treatment, for intestinal involvement.
Disclosure of Interest: None declared.

P680
Outcome and risk factors for pediatric patients receiving an haploidentical transplantation using CD3/CD19 depleted grafts

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Introduction: Haploidentical hematopoietic stem cell transplantation using T-cell depleted grafts (haploHSCT) is an option for pediatric patients with high-risk hematological malignancies lacking an HLA-identical donor.
Materials (or patients) and methods: Since December 2006 to December 2014; 65 children with hematological malignancies underwent a total of 67 haploHSCT using CD3+/CD19+ depletion for graft manipulation. Eighteen patients were in 1st CR, 25 in 2nd CR and 24 were in >2nd CR or persistent disease at time of transplantation. The conditionig regimen consisted of 30 mg/m2/d of i.v. fludarabine on days -6 to -2, 3.2-4.8 mg/kg/day of i.v. busulfan on days -6 and -4 and 5 mg/kg/day of i.v. thiotepa on days -3 to -2. PBPC were mobilized and collected in the standard manner. GvHD prophylaxis included CsA 3 mg/kg/day from day -1. Allografts contained a median of 7.29 x10^6 CD34 cells/kg and 1.0 x 10^4 CD3 cells/kg.
Results: Median times to neutrophil and platelet recovery were 13 and 10 days, respectively. The probability of aGvHD and cGvHD were 13 ± 5% and 31 ± 10% respectively. NRM was 14 ± 5% by day +100 and 21 ± 6% by 2 years after transplant. Cause of dead were relapse in 9 cases, severe viral infections in 7 and graft failure in 2. The probability of relapse was 29 ± 8%. With a median follow-up of 24 months, the probability of DFS was 55 ± 8%. On a multivariate analysis the factors that positive impact on DFS were age below 12 years (HR;0.26, 95%CI: 0.09-0.79 $P<0.009$) and cGvHD (HR;0.68, 95%CI: 0.01-0.53 $P<0.01$).
Conclusion: Our results suggest that haploidentical donors are a good option for pediatric patients with high-risk hematological malignancies who need an allogeneic transplantation. Graft manipulation resulted on low incidence of severe aGvHD. DFS was better for patients with cGvHD mainly due to lower relapse incidence. Severe viral infections is a relevant problem in the early phase after transplantation.
Disclosure of Interest: None declared.

P681
Unmanipulated haploidentical transplantation for childhood and adolescent severe aplastic anemia

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Introduction: In this study, we analyzed the results of allogeneic transplant from a haploidentical family donor in children and adolescent with severe aplastic anemia.
Materials (or patients) and methods: Between April 2011 and December 2014, 8 patients (SAA: 3, VSAA: 5) received unmanipulated PBSCT from haploidentical family donors. Median age at transplant was 14 years (range: 7.6-24.1). The conditioning regimen consisted of cyclophosphamide (25 mg/kg/day from D-5 to D-2), fludarabine (30 mg/kg/day from D-6 to D-2) and rabbit ATG (2.5 mg/kg/day, from D-4 to D-1). GVHD prophylaxis consisted of cyclosporine A and short term methotrexate.
Results: As of Dec. 2014, the median follow up duration of survivors was 29 months (range: 6-42). Median infused cell doses were as follows: MNC 21.3 x 10^8/kg, CD34+ 10.3 x 10^6/kg, CD3+ 59.8 x 10^7/kg. Median time to neutrophil and platelet engraftment was 12 days (range: 11-48) and 22 days (range: 14-157) respectively. Two patients showed primary graft failure and received a second transplantation. Six of 7 evaluable patients developed ≥ grade I acute GVHD (2 Gr II, 4 Gr I) and 5 patients developed limited chronic GVHD, all of which showed resolution with first-line immunosuppression. CMV DNAemia was positive in 5 of eight patients and resolved with preemptive ganciclovir. None of evaluable 7 surviving cases developed PTLD. One patient who failed to show engraftment died from pulmonary hemorrhage, resulting in 87.5% (7/8) overall survival. Median donor chimerism at last follow-up for the remaining patients was 99.5% (range: 99-100) with all showing transfusion independence.
Conclusion: The haploidentical transplantation without *in vitro* T cell depletion is a feasible option for children and adolescents with SAA who lack a suitable HLA-matched donor.
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P682

Outcome of 29 patients with Wiskott-Aldrich Syndrome: Single center experience

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Introduction: Wiskott-Aldrich syndrome is an X-linked disorder caused by mutations in the gene that encodes the Wiskott-Aldrich syndrome protein (WASp). The classic features of WAS include 1) immune deficiency due to both innate & adaptive immune defects & thus increased susceptibility to infections, 2) micro thrombocytopenia, and eczema (1,2). WAS is not as common in the Arabian peninsula as it is in the western world since the autosomal recessive types of Primary Immune Deficiency (PIDD) takes the lead in our region due to high rate of consengious marriages. WAS cases represents only 3-5% from all PIDD in our population, while Severe Combined Immune Deficiency (SCID) represents more than 60% of PIDD cases.
Materials (or patients) and methods: In this study we retrospectively analyzed our experience with transplantation in patients with WAS recorded in our data base till December 2013 at our tertiary center. Two families were excluded because of suspected WIP rather than WAS mutation.
Nineteen confirmed WAS patients have stem cell transplant,13 patients received transplant from HLA-identical matched related donors while 6 patients received HLA- matched unrelated cord blood stem cells. We did not perform any MUDD or T-cell depleted haploidentical stem cell transplant in our center on any of our patients.
Results: The overall survival of the transplanted patients was 89.5%, with 100% survival in the HLA-matched related stem cell recipients and 66.6% survival in the matched –unrelated Cord blood stem cell recipients. Survival among non-transplanted patients was only 60% till the end of the study with very poor quality of life.
Conclusion: According to our findings we recommend Hematopoietic stem cell transplantation in patients with WAS as early as possible if a matched related donor available, for those with no such donor, Cord blood hematopoietic stem cell represent a good alterative in these patients especially those with severe disease.
Disclosure of Interest: None declared.

P683

Brentuximab vedotin in heavily treated refractory or relapsed pediatric Hodgkin lymphoma patients who received autologuos stem cell transplantation(ASCT), a single Turkish centre study

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Introduction: Although Hodgkin Lymphoma(HL) is highly curable disease espacially in early stages,in advanced stage disease it becomes a big dilemma, when it is refractory or relapsed after several chemoterapy courses. Brentuximab vedotin (BV) is an antibody-drug conjugate that targets CD30-positive malignancies via an anti-CD30 monoclonal antibody linked to monomethyl auristatin E, a microtubule-disrupting agent, by a protease-cleavable linker. It has been shown in adult studies as effective salvage therapy for relapse after ASCT or for primary refractory HL.
Materials (or patients) and methods: We examined the effect of BV (1,8 mg/m2-every 3 weeks; 2- 8 courses) - AVD (Doxorubucin, Vinblastin, Dacarbazin; 2- 6 courses) combination treatment on six pediatric HL patients that have been severaly treated for refractory or ralapsed advanced stage HL, either before (5 patients) or after (one patient) ASCT (Between August 2012- November 2014). Results have been assesed according to patients clinical status, sedimantion rate, and PET results.
Results: One patient who received BV after his disease relapse 9 months later his ASCT was first pediatric patient who received BV in our centerand country. He was in remission with negative PET result after 4 couses BV- AVD (Alternating, Rituximab - ICE; R-ICE, due to his CD 20 positivity in tumor tissue). After 8 courses of BV he was able to performed MUD (Match unrelated Donor) SCT, and is doing well with no severe side effect. Other 5 pediatric HL patients were not able to get remission with any other classical HL chemotherapy protocols (but only one who showed early relapse just before her ASCT) received 2-6 courses of BV- AVD (some of them alternate with R-ICE, due to CD 20 positivity in their tumor tisue) and all were able to receive ASCT with negative PET imaging result and with normal sedimantation rate. There was no other side effects but only mild myelosuppression.
Conclusion: BV is a well tolarated and encouraging, effective targeted therapy especially when combined with AVD or alterned with R-ICE (when needed) for refractory or relapsed pediatric HL patients who planned to receive ASCT.
Disclosure of Interest: None declared.

P684

Effect of central nervous system complications and radiologic findings on patients' survival after hematopoietic stem cell transplantation

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Introduction: Hematopoietic stem cell transplantation (HSCT) is a curative treatment which has been increasingly used for the many malign and benign hematologic childhood disorders, metabolic and genetic diseases and immune deficiencies. It has been shown by many studies that central nervous system (CNS) complications are seen in 11-59% of the patients after HSCT. Early diagnosis, treatment and succesful management of the patients have remarkable effect on survival. Radiologic evaluation is one of the most important methods in early diagnosis for neurological complications.
Materials (or patients) and methods: We evaluated the relation between positive radiolgical findings and patient survival among patients who had HSCT in our institution. We aimed to provide information that may be helpful in decreasing HSCT related mortality and morbidity.
Results: We retrospectively evaluated records of all the patients who had HSCT between August 1998-January 2014. There were total 489 patients who had HSCT in this period and 91 (18,6%) of them had developed neurologic symptoms and neuroradiologic evaluation. Forty-seven (51,6%) patients had positive findings and 44 (48,3%) patients had no radiologic findings. Patients' ages, sexes, presence of neurologic finding before HSCT, intrathecal treatment history, CNS radiotherapy history had no significant effect on positive radiological findings. Mean duration between HSCT and appearance of neurologic symptoms was $87,4 \pm 14,4$ days. There was no significant effect of preparatory regimen, type of graft versus host disease prophylaxis, source of stem cells, and engraftment status on positive radiologic findings.Most frequent neurologic complaint was convulsions, which was seen on 34 (37,4%) cases, whereas most frequent radiologic finding was white matter lesions, which was seen on 22 (46,8%) cases. Posterior reversible encephalopathy syndrome (PRES)

accounted for 18 (81,8%) of all white matter lesions. When final neurologic diagnoses were considered, PRES was the most frequent (19,8%) and intracranial bleeding was the second (11%). In the patients who died after transplantation, the reason of death was also evaluated. In the patients with positive radiologic findings, the ratio of death due to neurologic complications was significantly higher than the patients with no radiologic findings ($P < 0,05$). The mean patient survival was $52,5 \pm 7,2$ months in patients who had HSCT and developed neurologic complications. Survivals were also evaluated according to presence of radiologic findings. In the group of patients with positive findings, mean survival was $30,5 \pm 4,5$ months, whereas in patients with no findings, mean survival was $68,6 \pm 10,1$ months. The presence of radiologic findings was significantly decreasing the mean survival ($P < 0,05$). When the findings were divided into three groups as cerebrovascular complications (bleeding, ischemia and infarction), white matter involvement (including PRES) and infections, the shortest mean survival was in the cerebrovascular complication group ($P < 0,05$).

Conclusion: Neurologic complications are not rare in patients who had HSCT. In these patients, prompt neuroradiologic evaluation is mandatory. In the management of these patients, it should always be kept in mind that positive neuroradiologic findings may considerably shorten the patient survival.

Disclosure of Interest: None declared.

P685

Transfer of *Ex Vivo* Expanded NK and γδT Cells from Untouched Posttransplant PBMCs to Clear Minimal Residual Disease in Acute Myeloid Leukemia

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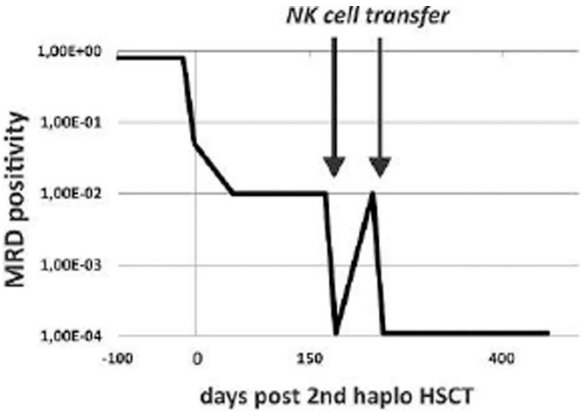
Introduction: GMP-grade NK cell expansion for clinical purpose has been demonstrated feasible and safe [1, 2]. Here we share our pilot data on posttransplant immunotherapy with *ex vivo* expanded NK cells and γδ T cells from PBMCs to treat minimal residual disease in a pediatric patient with relapsed myeloid leukemia.Our patient, a 13 year old boy who underwent 2nd allogeneic stem cell transplantation (haploidentical stem cell transplantation from his mother) due to posttransplant relapsed acute myeloid leukemia. After the 2nd haploidentical stem cell transplantation (SCT) minimal residual disease (MRD) was detected by multiparameter flow cytometry and by two molecular markers CALM-AF10 fusion transcript and a NRAS-mutation.

Materials (or patients) and methods: For posttransplant compassionate use immunotherapy by NK cell transfer, NK cells were expanded from untouched isolated PBMCs of the patient post 2nd haploidentical SCT. GMP-grade expansion of the NK cells was done under static conditions in our GMP-facility. Isolated PBMCs were pooled with 100 Gy irradiated K562mb15 4-1BBL feeder cells (kindly provided by Dario Campana) in a proportion of 1:20 (NK to K562mb15 4-1BBL) [2]. Cells were seeded in conventional cell culture flasks ($175cm^2$) at a density of 1.1E6 cells/ml. Media contained RPMI1640 supplemented with 10% AB-human serum, 1% L-glutamine and 100IU Proleukine® IL2/ml. Daily monitoring for cell number, white blood cell differentiation, pH of the cell culture, glucose metabolism, lactate production and microbial sterility testing at the beginning and the end of the expansion period.

Results: The cell product was harvested on day 15-17. Fresh isolated PBMCs and the expanded NK cell product were characterized by flow cytometry. NK cells were expanded >1000 fold (3.1 and 3.4 log-fold) in 14-17 days. The product contained a total number of 9.8E9 and 19.9E9 cells, which was 328 and 665E6/kgBW. The expansion protocol supports NK and γδ T cell expansion whereas the number of αβ T cells stays



stable. Cytotoxicity assay against various targets revealed excellent cellular cytotoxicity and antibody dependent cellular cytotoxicity. To prevent relapse in our patient with post-transplant MRD positivity, NK cells from the patient post 2nd haploidentical SCT were expanded for cellular immunotherapy. 2 weeks post 1st NK cell transfer (day +170) the patient achieved complete MRD response in the bone marrow. Unfortunately the patient again showed detectable MRD one month later. Therefore another NK cell expansion and transfer was done. 2 weeks post 2nd NK cell transfer (day +232) the patient again achieved complete MRD response in the bone marrow and is in complete molecular remission ever since (day +460). The NK cell products were tolerated well. Transient coughing and temporary increase of temperature were registered.

Conclusion: Both, *in vitro* and *in vivo* effect of the NK cell product were documented. Clinical use of expanded and activated NK cells and γδ T cells can induce molecular remission in posttransplant MRD positive acute myeloid leukemia.

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Disclosure of Interest: None declared.

P686

Use of TCRαβ/CD19 depletion results in superior immune recovery compared to CD34 selection after transplantation of haploidentical peripheral stem cells in children

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Introduction: Transplantation of haploidentical stem cells has become an accepted option for pediatric patients and adults with high risk malignancies who lack a matched related or unrelated donor. In recent years, the majority of pediatric transplant centers chose the CD34 positive selection of peripheral stem cells, which allowed minimizing GvHD by effective reduction of T-cells in the graft. However, infectious complications caused by delayed immune recovery were a major reason for transplant related mortality (TRM).

Materials (or patients) and methods: In order to improve the immune recovery, we have established a new T-cell depletion method which removes αβ+ T-lymphocytes via a biotinylated

anti-TcR$\alpha\beta$ antibody followed by an anti-biotin antibody conjugated to magnetic microbeads while retaining $\gamma\delta+$ T-lymphocytes, natural killer (NK) cells and other cells in the graft. In addition, CD19+ B-lymphocytes were concomitantly depleted for the prevention of post-transplant EBV-associated lymphoproliferative disease.
Immune recovery was retrospectively analyzed in a cohort of 41 patients with acute leukemia, MDS and non-malignant diseases, who received $\alpha\beta$ T- and B-cell depleted allografts from haploidentical family donors. Conditioning regimens consisted of fludarabine or clofarabine, thiotepa, melphalan and serotherapy with OKT3 or ATG-Fresenius®. Graft manipulation was carried out with anti TCR$\alpha\beta$ and anti CD19 antibodies and immunomagnetic microbeads. $\gamma\delta$ T-cells and NK-cells remained in the grafts.
Results: Primary engraftment occurred in 88%, acute graft versus host disease (aGvHD) grade II and III-IV occurred in 10% and 15%. Immune recovery data were available in 26 patients and comparable after OKT3 ($n=7$) or ATG-F® ($n=19$). Median time to reach >100 CD3+ cells/μl, >200 CD19+ cells/μl and >200 CD56+ cells/μl for the whole group was 13, 127 and 12.5 days. Compared to a historical control group of patients with CD34 positive selected grafts, significantly higher cell numbers were found for CD3+ cells at days +30 and +90 (267 vs. 27 and 397 vs. 163 cells/μl), for CD3+4+ cells at day +30 (58 vs. 11 cells/μl) and for CD56+ cells at day +14 (622 vs. 27 cells/μl).
Conclusion: The clinical impact of this accelerated immune recovery will be evaluated in an ongoing prospective multicenter trial.
Disclosure of Interest: None declared.

P687

Contribution of reiterated therapeutic drug monitoring (TDM) of iv busulfan dosing in infants and older children undergoing hematopoietic stem-cell transplantation (HSCT) to better control the targeted exposure to a major alkylating agent

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Introduction: Busulfan (Bu) is the standard backbone for HSCT conditioning regimens (CR). It has a narrow therapeutic window (TW) and graft rejection or toxicity are related to Bu exposure. In infants, Bu exhibits large pharmacokinetic (PK) variability and its clearance is non-linearly related to body weight (BW). Bu dosage is stratified according to BW and commonly monitored once after the 1st of 16 total 2-hr infusions (96-hr exposure). To optimize Bu duration/intensity exposure in children undergoing HSCT, we studied the possible contribution of double TDM of IV Bu in children receiving Bu-based CR for HSCT, by comparing the expected and calculated exposure values after performing 1 PK (1st dose, PK1) and finally 2 PK (1st dose, PK1, and 9th dose, PK2).
Materials (or patients) and methods: In this single-centre observational study (05/2012-09/2014), 38 patients (Pts) receiving Bu-based myeloablative CR for HSCT were prospectively included with median follow up of 16 months [3-31]. Median age was 16.5 months [1-193] and BW 11.6 kg [3-59]. Most Pts had non-malignant diseases, received allogenic HSCT and Bu-based CR in combination with Flu. The 1st dose and the tight and high TW were set up according to EBMT-ESID recommendations [ΣAUC = 20,706-23,180 μmol/L*min]. Bu PK was assessed on 3 plasma samples/PK (LC-MS^2 analysis) with area under the concentration-time curve (AUC) calculation from the 1st and 9th doses, using the NONMEN® software. AUC calculated after the 1st dose and extrapolated to the 16th one were compared by a Wilcoxon signed-rank test to the expected AUC and to the sum of $AUC_{1\text{-}6}$ with TDM1 applied from the 7th dose with or without TDM2 applied from the 14th dose; $p \leq 0.025$ was considered statistically significant.
Results: A median Bu posology of 1.16 mg/kg x 4/day for 4 days was given. We demonstrate that: (a) estimated total AUC obtained from 2 PK differ significantly from those calculated after none ($P=1.196$ E-4) or PK1 alone ($P=6.377$ E-6), (b) double TDM allows achieving no difference between the expected AUC desired by the medical staff in view of diseases vs. estimated AUC ($P=0.683$). In 1/38 Pts, PK did not lead to change Bu dosage. In 5/38 and 10/38 Pts, dosage was modified after PK1 or PK2 alone (TDM1 Group), respectively. In 22/38 Pts, changes were required twice, after the 2 PK (Double-TDM Group). The median total doses of Bu were as follows: (a) a theoretical value of 235.2 mg (20.1 mg/kg) would have been given without TDM (16 doses), (b) 215.1 mg (18.2 mg/kg) were administered in the TDM1 Group (16 doses), (c) 237.6 mg (19.5 mg/kg) were administered in the Double-TDM Group, avoiding the infusion of 27 mg vs. PK1 alone in these Pts. In 11/38 (28.9%) Pts, a decision of discontinuation of Bu exposure was taken as the target desired by the medical staff was achieved after 13 (4 Pts) or 14 (7 Pts) doses; the mean total amounts of Bu were 283.4 mg and 268.1 mg, respectively, vs. 349.6 mg and 313.5 mg theoretically after TDM1 alone.
Conclusion: In this paediatric series, the double TDM of Bu is a relevant and feasible option to better achieve a narrow TW and to potentially minimize the risk of overexposure. Correlations with toxicities (VOD, aGVH), CR, OS are under analysis. Based on these data, the double-TDM procedure is routinely applied in our centre.
Disclosure of Interest: None declared.

P688

A Single Center Experience of Haploidentical Stem Cell Transplants in Pediatric Leukemia

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Introduction: Between 2011 and 2014, haploidentical stem cell transplants (SCT) from parental donors in 14 pediatric patients with leukemia who lack suitable HLA matched donors were performed in our program.
Materials (or patients) and methods: Half (43%) of these patients (8 acute lymphoblastic leukemia, 3 acute myeloid leukemia (AML), 2 therapy-related AML [t-AML]; 1 myelodysplastic syndrome/ refractory anemia with excess blasts [MDS/RAEB]) had active disease at the time of SCT; 2 of them had 1 or 2 previous SCT. All, except the earliest patient who had t-AML received T cell depleted peripheral blood SCT (3 with CD3 depletion; 6 with TCRab depletion; 4 with CD45RA depletion). Radiation based (TBI or TLI); reduced toxicity preparative regimen was used in 12 patients while 2 infants received ATG (Thymoglobulin) or Alemtuzumab in place of radiation.
Results: Primary engraftment occurred at a median of 11 (8 to 24) days in all patients. 3 patients subsequently experienced graft failure associated with acute rise of LDH (median peak of 1011 (range, 813 to 1915) U/L at a median of 17 (range, 13 o 17) days; 2 of whom had active disease (1 AML; 1 MDS/ RAEB) at SCT and the 3rd was an infant who received Alemtuzumab / chemotherapy at infant dosing (per kg). All 3 patients were successfully rescued with T-replete alternative haploidentical donor bone marrow transplants after reduced intensity conditioning (RIC) and post-transplant cyclophosphamide (pCy). Mild (grade I-II) acute and chronic graft-versus-host disease (GVHD) occurred in 18% and 9% of evaluable patients, respectively. None had grade III-IV acute GVHD or extensive GVHD. 100- day regimen-related mortality occurred in 1

patient (7%). Overall survival / disease free survival were: 71% / 36% (whole group); 75% / 75% (in remission) versus 67% / 17% (active disease). 83% (5 of 6) of patients with active disease at time of SCT relapsed at a median of 82 (31 to 176) days; all of whom received salvage cell therapy subsequently. Only 1 of 8 patients who entered SCT in remission relapsed; this patient had t-AML who received a RIC/pCy regimen.

Conclusion: Similar to the experience of Lang P. et. al. (BJH 2014), patients in remission fared significantly better than those with active disease at SCT. The role of peri-SCT cell therapy should be investigated to improve results in very high risk patients.

References: Lang P. et al. British Journal of Haematology, 2014, 165, 688–698

Disclosure of Interest: None declared.

P689

The Father, the Son and Stem Cells: TCR$\alpha\beta$ and CD19 depleted Haploidentical stem cell transplant in Hoyeraal - Hreidarsson Syndrome

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Introduction: Hoyeraal-Hreidarsson syndrome (HHS) is an extremely rare telomere biology disorder representing the most severe spectrum of dyskeratosis congenita (DKC). Stem cell transplant is the only known cure for marrow failure associated with DKC. Conditioning chemotherapy toxicity is significant hence reduced intensity conditioning (RIC) is recommended. Graft manipulation with TCR$\alpha\beta$ and CD 19 depletion is a novel technique of Haploidentical stem cell transplantation, which has recently shown promising results in non-malignant paediatric stem cell transplantation.

Materials (or patients) and methods: We describe an infant who presented with prematurity, intrauterine growth retardation and mild neonatal thrombocytopenia that rapidly progressed to severe bone marrow failure. Combination of these features with microcephaly, developmental delay, severe cerebellar hypoplasia and short telomere length confirmed a diagnosis of HHS. Mutation analysis for known genes is negative. We are awaiting the results of whole exome sequencing. We proceeded for haploidentical transplantation in view of severe pancytopenia needing frequent blood product infusions and lack of a suitable donor.

Results: Haploidentical transplant was performed at 10 months of age using father as a donor (KIR mismatched and KIR B haplotype). RIC chemotherapy with ATG (Thymoglobulin, 2.5 mg/kg/day X 4 days), Fludarabine (40 mg/m^2/day X 4 days) and Treosulfan (12 gram/m^2/day X 3days) was used. GCSF mobilized peripheral blood stem cell was collected and processed by negative selection for TCR$\alpha\beta$ and CD19. Infused product had CD34+ cells 36 X 10^6/kg and TCR$\alpha\beta$ cells 0.45 X 10^4/kg. No graft versus host disease (GVHD) prophylaxis was used. Rapid platelet engraftment on D+11 and neutrophil engraftment on D+15 were noted. Apart from mild mucositis, one episode of culture negative fever and poor feeding no major complications were observed. He is now 7 weeks post-transplant with no infections or GVHD. Day 30 lymphocyte subset analysis shows excellent NK cell reconstitution and 100% donor chimerism.

Conclusion: TCR$\alpha\beta$ and CD19 depleted Haploidentical stem cell transplantation when combined with RIC offers a useful strategy in children with DKC who are otherwise considered a high risk group for transplant related toxicity.

Disclosure of Interest: None declared.

P690

Successful Hematopoietic Stem Cell Transplantation In Autosomal Recessive Hyper-IgE Syndrome Due To DOCK8 Deficiency: Single Center Experience

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Introduction: The autosomal-recessive variant of hyper-IgE syndrome (AR-HIES) is a combined immunodeficiency characterized by high serum IgE, eosinophilia, atopy, recurrent sinopulmonary and skin infections, molluscum contagiosum, human papillomavirus and HSVs. Here, we report six patients with AR-HIES due to DOCK8 deficiency undergoing successful hematopoietic stem cell transplantation (HSCT).

Materials (or patients) and methods: Patients were between 6 and 8 years old at the time of transplantation. Four patients were male and two patients were fenale. All patients had skin involvement with molluscum contagiosum, recurrent sinopulmonary infections. In addition, one patient had coombs positive hemolytic anemia, one patient had acute disseminated encephalomyelitis (ADEM) and one patient had emphysema in high-resolution computed tomography. Donors of two patients were HLA matched siblings. Other four patients had unrelated donors having 9/10 or 10/10 HLA matching. Stem cell source is bone marrow in four and peripheral stem cell in two. Conditioning regimen consisted of busulfan and fludarabine with/without ATG and GVHD prophylaxis consisted of cyclosporine with/without methotrexate. Total nucleated cell count in bone marrow transplants ranged between 3.5x10^8/kg whereas CD34 count was 6x10^6/kg in two peripheral stem cell transplants. All patients had successful engraftment with neutrophil recovery (neutrophil count $>500/mm^3$) day ranged between +13 and +22. Donor chimerism achieved at the end of one month in all patients. In the first 100 days, one patient had CMV reactivation, two patients had acute GVHD and one patient had probable invasive fungal infection. In patient with ADEM, progression in the post-transplantation period required steroid therapy. The patient had stable neurologic disease in spite of steroid dependency at the end of five months after transplantation. After 100 days, one patient had chronic limited GVHD requiring immunsupresssive treatment even in the seventeenth month after transplantation. Follow-up time ranged between 100 days and 17 months. All patients are alive with full donor chimerism and with no disease associated with the primary diagnosis.

Results: There is a paucity of data regarding the role of allogeneic HSCT in this disorder except case reports. This is the first report containing six patients with DOCK8 deficiency who underwent HSCT. This experience demonstrates that HSCT with combination of conditioning with busulfan and fludarabine may be potentially curative in DOCK8 deficiency with less toxicity and relatively less complications.

Conclusion: These results suggest that HSCT may be an option to treat DOCK8 deficiency. Furthermore, early diagnosis of this newly discovered immune deficiency prior to life-threatening complications likely optimize clinical outcomes following HSCT. Long-term follow-up will be needed to determine whether correction of the hematopoietic compartment is sufficient to protect DOCK8 deficient.

Disclosure of Interest: None declared.

P691
National Pediatric Hematopoietic Stem Cell Transplantation Activity in Turkey

(on behalf of Turkish Society of Pediatric Hematology, SCT Study Group)
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Introduction: Twenty pediatric stem cell transplantation (SCT) centers have been active in our country since 1988. The Pediatric SCT Study Group in the Turkish Society of Pediatric Hematology has determined SCT indications at the end of regular meetings since 2000 and offered them to hematology-oncology specialists. In 2007, "online access database'' was started to record all pediatric transplant data in our country. By this database, transplant centers can register and update their own data and use the recorded data for statistical analysis.
Materials (or patients) and methods: According to the analysis carried out in December 2014, 3798 pediatric transplants (allogeneic 85.7%, autologous 14.3%) were registered in the database. Mean age of the data was 7.9 ± 5.2 years, median age was 7 years (1 month-22years), male-female ratio was 1.53. The number of transplants registered in 2012 and 2013 were 539 and 595 respectively. In 2007 "online access database'' was started to record all pediatric transplant data in our country.
Results: The number of unrelated and allogeneic transplants as well as the total number has increased steadily in recent years. Nearly half of the pediatric transplants in our country have been carried out in the last 4 years.
Non-malignant diseases such as hemoglobinopathies, immuno-defficiencies, aplastic anemias, metabolic diseases and osteopetrosis were 50.8% of all pediatric transplants. Stem cell sources in entire group were bone marrow (53.6%), peripheral stem cell (38.2%) and umbilical cord blood (6.4%). Donors in allogeneic transplants were matched siblings in 61.7%, parents in 16.4%, related donors in 4.0% and unrelated donors in 17.2%.
In 559 transplants, the donor was unrelated. Stem cell sources in this group were bone marrow (46.0%), cord blood (25.4%) and peripheral stem cell (28.6%). Ten of ten, 9/10 and 8/10 HLA matched transplantations were 38.6%, 33.8% and 0.9% respectively. 71% of the entire unrelated donor transplants have been performed within the last 3 years. According to the national database records, 64.8% of all patients with unrelated donor transplants are alive.
In addition, 55.9**%** of the 227 haploidentical transplants were immunocompromised patients. 90.6% of 765 transplants in thalassemia major group are alive. Reconstitution or aplasia after transplantation was seen in 11.1%.
Neuroblastoma is important in transplantation practice as it benefits from autologous transplantation. 61% of 213 advanced stage neuroblastomas with autologous transplants are still being monitored according to our registry. Hodgkin's lymphoma is another disease that benefits from autologous transplantation.
In the twenty-five-year period with SPSS 16.0-Kaplan-Meier survival analysis, overall survival in allogeneic transplants, autologous transplants and in entire group are 75.6%, 63.9% and 73.9% respectively.
Conclusion: Consequently, pediatric bone marrow SCT with a history of more than 25 years in our country has reached a significant number of patients today. Also half of the transplantations in the registry have been performed in the last 4 years. Europian standards in pediatric transplantation have also been reached along with the increase in the number of transplantation centers and the capacity of the existing ones. Therefore, the assessment of future years will provide more accurate information.
Disclosure of Interest: None declared.

P692
Evaluation of Respiratory and Cardiovascular Complications of Hematopoietic Stem Cell Transplantation In Children

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Introduction: Hematopoietic stem cell transplantation (HSCT), is the main curative treatment for many diseases. Although an increase of survival rate provided, complications that may impair the quality of life and development. Respiratuar and cardiovascular complications are very important for mortality and morbidity and also depend on risk factors such as chemotherapy, radiotherapy, age of patient, patients' health status before HSCT, primer illness, receive chemotherapy and / or radiotherapy before HSCT. These complications may affect adversely survival, therefore patients should be followed closely transplant and post transplant period.
In this study, we aimed to determine the cardiovascular and respiratory complications, incidence and risk factors of HSCT
Materials (or patients) and methods: 40 patients older than 5 years who have undergone HSCT in Ege University Medicine Faculty, Pediatric Stem Cell Transplantation Unit between 2012 and 2013 were planned to be followed for 1 year prospectively. Pulmonary function tests (PFT) and echocardiography were performed pre HSCT, on month after $+1$, 3, 6, 12. Risk factors for complications such as demographics, HSCT characteristics, primary diagnosis, donor properties, conditioning regimen, total body irradiation (TBI), graft versus host disease (GVHD), CMV seropositivity, hypertension, diabetes mellitus, hyperlipidemia were recorded.
Results: 45% female, 55% male had 136 months median age. 97,5% of cases were undergo allogenic HCST while 2,5% were autologous HSCT. Primary diagnosis were malignancy (47,5%), bone marrow failure (20%), immunodeficiency (17,5%), hemoglobinopathies (10%), metabolic disease (5%). Unrelated stem cell donors was 52.5%, 45% was related donor. Myeloablative treatment and non-myeloablative treatment were given to 60% of and 40% of cases, respectively. During follow up, acute GVHD was observed in 27,5% of cases, chronic GVHD was observed in 5% of cases. Bronchiolitis obliterans (BO) was seen only as the respiratuary complications and observed only in two patients that's incidence was % 5,7. BO was found to be statistically correlated with acute GVHD ($P = 0{,}018$). There wasn't any significant relationship between respiratuar complications and the other risk factors. All of except 2 patients were followed with normal PFT parameters during 1 year. When compared the mean PFT measurements between pre-HSCT and $+1$. year, statistically significant difference wasn't

observed ($P > 0,05$). We found that the our incidence of BO was lower than literature. None of the patients experienced significant cardiac disorder, such as systolic and diastolic dysfunction, in serial echocardiyographic imaging.
Conclusion: There are rare number of studies on the respiratory and circulatory complications after pediatric HSCT, therewithal all of these studies are retrospective. This prospective study aimed to clarify these complications in our clinic. The rate of complications were found significantly lower in our study. This can be explained by tailored conditioning regimen for each patient and follow-up at frequent intervals.
Disclosure of Interest: None declared.

P693
Outcome of allogeneic hematopoietic stem cell transplantation in pediatric patients with chronic myeloid leukemia: a multicenter study by the Turkish Pediatric Bone Marrow Transplantation Study Group (TPBMT-SG)
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Introduction: Chronic myeloid leukemia (CML) is a rare disorder in children. They have longer life expectancies than adults; the goal of CML treatment in children should be cure rather than disease suppression. Because of the possibility of decades-long tyrosine kinase inhibitor (TKI) treatment, which also occurs during periods of active growth, morbidity related to TKI therapy for CML is different in children than in adults. On top of all these, the role of hematopoietic stem cell transplantation (SCT) is still not clear in pediatric patients with CML. The aim of the study was to determine the outcome (mortality, relapse and long term survival) of children with CML who received allogeneic stem cell transplantation in Turkey
Materials (or patients) and methods: We retrospectively analyzed the data of 42 children with CML who received 45 allogeneic SCT at 7 pediatric centers in Turkey between January 2000 and August 2014.
Results: Twenty-three boys and 19 girls with CML whose ages ranged between 2-21 years (median 13 years, 24% < 10 years). SCT was performed in first and second chronic phase (CP) in 34 (81%) and 8 (19%) of the patients respectively. The median time to transplant from the diagnosis was 13 months (4–60 months) in first CP and 22 months (12 -66 months) in second CP. 95% of the patients received TKI's for median 12 months and complete or major molecular response was detected 50% of all patients prior to allogeneic SCT. The donor was HLA matched related (MRD) in 23 (55%) (20 matched sibling, 3 related family donor), matched unrelated (MUD) in 18 (43%) and mismatched related in 1 (2%) patients. Busulphan based chemotherapy were used for conditioning (+ cyclophosphamide or fludarabine) in 93% of the patients. Graft source was bone marrow in 27 (64%), peripheral stem cells in 14 (34%) and cord blood in 1 (2%) patients. Graft versus host disease (GVHD) prophylaxis was with cyclosporine alone or combined in all patients. Two patients underwent second transplantation from the same donor because of engraftment failure and the other after the relapse. Grade III/IV acute and extensive chronic GVHD were seen 19% and 14% of patients respectively. The median follow up was 25 months for patients in first CP and complete molecular remission was obtained 88% of them. The 5 year estimate of overall survival (OS) and event free survival (EFS) for the whole cohort was 66% and 63% respectively. EFS was 80% and 50% when the disease was early (first CP) and advanced (second CP) ($P = 0,015$). Transplant related mortality was 18%. Disease relapsed in four patients but only one of them transplanted in first CP.
Conclusion: SCT is a curative therapy for pediatric CML patients especially in early phase of disease. Transplant related mortality is higher than desirable and long term outcome is required for our patients. There is a need for standardization of the SCT procedure and also we need more information about the curability and morbidity of TKI's.
Disclosure of Interest: None declared.

P694
Histologic features of intestinal thrombotic microangiopathy in patients with high risk TMA after HSCT
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Introduction: Our group recently showed that high risk transplant-associated thrombotic microangiopathy (TMA) can present with multisystem involvement and has poor outcome after HSCT with <20% 1-year survival (Jodele, Blood 2014). TMA may involve intestinal vasculature and can present with bleeding and ischemic colitis. There are no established pathologic criteria for diagnosis of intestinal TMA (iTMA). The goal of our study was to review available tissue specimens obtained after HSCT in order to identify histologic features of iTMA.
Materials (or patients) and methods: Fifty consecutive HSCT patients who underwent endoscopy for gastrointestinal symptoms were evaluated for histopathologic signs of iTMA using 8 histologic criteria described in literature: mucosal hemorrhages, loss of glands, schistocytes, fibrinoid debris in the vessel lumen, intravascular microthrombi, endothelial cell swelling, endothelial cell separation and total denudation of mucosa. The reviewing pathologist was blinded to patients' clinical history. Histologic markers were listed as present or absent. For patients having multiple evaluations after HSCT,

[P694]

Table: Histologic features in patients with TMA and without

HSCT patients with gastrointestinal symptoms (n=50)				
	TMA/iGVDH n=15	No TMA/iGVDH n=21	No TMA/no iGVDH n=14	p=value
Mucosal hemorrhages	12 (80%)	11 (52.4%)	5 (36%)	0.057
Loss of glands	11 (73.3%)	8 (38%)	0 (0%)	<0.0001
Intravascular schistocytes	10 (66.7%)	5 (23.8%)	3 (21.4%)	0.016
Intravascular fibrinoid debris	8 (53.3%)	2 (9.55%)	2 (14%)	0.008
Intravascular microthrombi	4 (26.6%)	0 (0%)	0 (0%)	0.010
Endothelial cell swelling	13 (86.6%)	13 (61.9%)	7 (50%)	0.086
Endothelial cell separation	8 (53.3%)	3 (14.3%)	1 (7%)	0.007
Total denudation of mucosa	7 (46.7%)	3 (14.3%)	0 (0%)	0.005

the first diagnostic tissue sample was used for this study. Patients were divided into 3 clinical groups based on the presence or absence of systemic TMA and intestinal graft versus host disease (iGVHD): TMA/iGVHD, no TMA/iGVHD, and noTMA/no iGVHD. Systemic TMA was diagnosed using rigorous clinical and diagnostic criteria. Comparison among the groups was done using Fisher exact test.

Results: Thirty percent of evaluated patients (15 of 50) had a clinical diagnosis of systemic TMA. Out of 35 patients without TMA, 21 had clinical and histologic evidence of gut GVHD while 14 did not. Incidence of stage 3-4 gut GVHD was similar in the TMA/iGVHD and noTMA/iGVHD groups (79% vs 64%). All histologic signs of iTMA except for mucosal hemorrhages and endothelial cell swelling were significantly more common in patients with systemic TMA ($P < 0.05$). Intravascular thrombi were exclusively seen only in patients with systemic TMA (Table).

Conclusion: We identified histologic features of iTMA that can be used to delineate vascular injury of the bowel in patients with TMA after HSCT. Recognition of these histological signs in patients with gastrointestinal symptoms after HSCT may guide clinical decisions.

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P695

Two Questions about the Use of NIH Scoring in Chronic GvHD? Applicability in Pediatric Age Group and Interpretation of the Scores

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Introduction: Chronic GvHD is the most common cause of non-relapse morbidity and mortality in allogeneic HSCT. In 2005, NIH published the diagnostic and scoring criteria for chronic GvHD and revised in 2014 (unpublished). The severity of chronic GvHD was stratified as mild (1-2 organs involved, other than lungs, maximum score 1 from each organ), moderate ($\geq$1 organ involvement with maximum 2 scores/organ or $\geq$3 organs with maximum score 1/organ or lung score of 1) or severe (3 in any organ or lung score of 2 or 3). Herein, we assessed the NIH scores and outcomes of a series of pediatric patients with chronic GvHD and questioned the applicability of the scoring system in children.

Materials (or patients) and methods: Between January 2007 and December 2014, a total of 167 pediatric patients underwent allo-HSCT and 16 (9.6%) developed chronic GvHD. These patients were diagnosed and scored according to NIH. The median age at HSCT was 9 years (1.5-17). BMT indications were AML ($n = 4$), WAS ($n = 2$), MDS ($n = 2$) and one patient from each as, JMML, beta-thalassemia major, chronic granulomatous disease, Griscelli, CML, ALL, ALD, Fanconi anemia. Of the 15 related donors, 2 (13%) were HLA mis-matched, stem cell source was PBSC in 10 (66%).

Results: The clinical data were summarized i

Conclusion: The mortality rate of chronic GvHD in our series was found as 6.3%, however the morbidities being significant. The severely stratified patients according to NIH had worse outcome; however patients with moderate chronic GvHD, such as patients 1 and 9, had clinically severe disease. Moderately classified patients may further be classified as mild to moderate and moderate to severe. Additionally, although this scoring system is efficient to use at presentation of chronic GvHD, the unresponsiveness to treatment may also be added in follow-up scores.

Disclosure of Interest: None declared.

P696

Serum growth factors (IGF-I and IGFBP-2) prior and after pediatric stem cell transplantation

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Introduction: Insulin-like growth factor 1(IGF-1), belongs to a family of peptides involved in the proliferation and differentiation of cells. IGF binding protein 2 (IGFBP-2) was

[P695]

Table 1. Clinical data of the patients

Patient	*NIH score*	*Organs involved (score for each organ)*	*NIH Severity*	*Treatment*	*Outcome*
1	3	PS(1)/Skin(1)/Joint (1)	Moderate	STE,TACRO,MMF,PHOTOP.,IMAT	Steroid related cataract, osteoporosis, fracture,GR,PR,Alive
2	3	Skin(1)/Lung (1)Eye(1)PS(1)/	Moderate	STE,MMF,PHOTOP.	Catheter infection,GR,CR,Alive
3	2	Lung (1)	Moderate	STE,MMF,IMAT,PHOTOP.	Catheter infection and thrombosis,PR,Alive
4	6	PS(1)/Skin(3)/Mouth(2)	Severe	STE,MMF,TACRO,PHOTOP.,IMAT	Catheter infection,G,PR,Alive
5	3	Myasthenia gravis (3)	Severe	STE,MMF,PE,Rituximab	Mechanical ventilation, CR Alive
6	3	PS (1) Skin (1) Lung (1)	Moderate	STE,CsA,MMF	Premature graying of hair, ON,PR,Alive
7	5	Mouth(1)/GI(2)/Liver(2)	Moderate	STE,MMF	ON,PR,Alive
8	3	GI(2)/Mouth(1)	Moderate	STE	PR,Alive
9	6	Skin(2)/Mouth (1)joint(1)/ Genital (2)	Moderate	STE MMF	Surgery,recurrent UTI,GR,PR,Alive
10	3	Skin(1)/Mouth (1)/Eye (1)	Moderate	STE,MMF,dasatinib(for CML)	PR,Alive
11	2	Liver(2)	Moderate	STE,MMF	GR,PR,Alive
12	1	Lung(1)	Moderate	STE,CsA,MF	GR,PR,Alive
13	8	PS(3),Eye(2),Lung(3)	Severe	STE,MMF,CsA(eye)	Respiratory failure, 2^{o} malignancy,No response,Exitus
14	2	Skin(2)	Moderate	STE,MMF,Calcitriol,Topical	CR,Alive
15	3	Skin(3)	Severe	TACRO	GR,PR,Alive
16	1	Mouth(1)	Mild	STE,MMF,Topical TACRO Topical TACRO	CR,Alive

PS: Performance score GR: growth retardation PR: partial response CR: complete response ON: osteonecrosis STE: steroid MMF: mycophenolate mofetyl TACRO: tacrolimus IMAT: imatinib PHOTOP.: photopheresis PE: plasma exchange n Table 1.

identified as an extrinsic factor that supports the survival and cycling activity of hematopoietic stem cells and critical peptide that promotes the survival and migration of acute leukemia cells. The goal of the study was determination of both peptides concentrations profile in children treated with stem cell transplantation.

Materials (or patients) and methods: Plasma IGF-1 and IGFBP-2 concentrations were measured using ELISA in fasting state in 19 children 1.5-19 (average 9) years old, 15 boys and 4 girls, referred to allogeneic haematopoietic stem cell transplantation (HSCT) due to neoplastic-74% (ALL-8, AML-5, MDS-1) and non-neoplastic-26% (CGD-2, SAA-1, ALPS-1, HiperIgM-1) diseases. HSCT group was studied twice – before conditioning and 8 months in average after transplantation. HSCT types were as follows: MUD-11, MSD-7, MFD-1. Two groups were recruited as a control: healthy group consisted of 21 children 4.3-17 (average 12) years old, 9 boys and 12 girls; obesity group including 27 children 4-18 (average 14) years old, 12 boys and 15 girls. Due to lack of enough plasma IGF1 study was not performed in healthy control.

Results: Comparing to healthy control (HC) and obesity (OB) concentration of IGFBP-2 was significantly higher in both HSCT subgroups. Median values of IGFBP-2 concentrations prior to HSCT, after HSCT, in HC and OB groups were as follows: 180; 254; 94 and 67.4 ng/ml respectively. In neoplastic diseases prior and after HSCT median concentrations were 175 and 255 ng/ml, in non-neoplastic subgroups 183 and 77 respectively. The differences were significant for all comparisons between neoplastic diseases subgroups and HC/OB. The same comparisons for non-neoplastic diseases gave not significant results. Median concentrations of IGF-1 were lower after then prior HSCT (97 vs. 147ng/ml, $P = 0{,}09$), and lower ($P < 0.001$) in all HSCT subgroups in comparison with OB (246ng/ml). Within median follow-up of 4 years 2 patients died due to relapse (AML and MDS; IGFBP-2 after HSCT 679 and 174ng/ml respectively.

Conclusion: As it was shown in the literature children with acute leukemia show increased IGFBP-2 and decreased IGF-I levels, what may be associated with the increased proliferation rate of transplanted bone marrow. The same profile was observed in our cohort. Besides IGFBP-2 play an important role in tumor proliferation and its elevated level was associated with high relapse risk after HSCT in childhood AML. Our patient with AML who died had 2,7 times higher IGFBP-2 concentration comparing to the median level observed in neoplastic diseases group after HSCT. Interestingly elevated level of IGFBP-2 was not observed after HSCT in non-neoplastic diseases (median values similar to controls) suggesting lower cell renewal rate in that group. Contrary to obesity IGF-1 levels were lower in both HSCT groups and decreased after transplantation suggesting lack of influence of IGF-1 on regeneration process. In conclusion increased IGFBP-2 is associated with high proliferation rate in children with acute leukemia after HSCT but not in non-neoplastic diseases. Leukemia patients with elevated IGFBP-2 level after HSCT should be carefully followed due to high risk of relapse.

Disclosure of Interest: None declared.

P698

Targeted busulfan conditioning and unrelated cord blood transplantation in pediatric primary immunodeficiencies and neurometabolic diseases other than Hurler's disease

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Introduction: Fludarabine-based conditioning combined with targeted myeloablative busulfan exposures (cumulative AUC: 80-95 mg/Lxh) is a promising treatment modality in patients with M. Hurler transplanted with unrelated cord blood (UCBT). In other non-malignant diseases (NMD) the experience with targeted busulfan and UCBT is scarce.

Materials (or patients) and methods: Between 2009 and 2014, $n = 11$ patients (age 0.4-7 yrs; median: 2.75) with NMD (primary immunodeficiencies: CGD $n = 2$, congenital neutropenia $n = 1$, ADA/IL7R/HLH $n = 3$, CHH $n = 2$; neurometabolic disease: X-ALD/Fucosidosis/alpha-Mannosidosis $n = 3$) were treated with high-dose fludarabine 180 mg/sqm (< 9 kg 6 x 1.2 mg/kg) (d-8 to -3), targeted i.v. busulfan (d-5 to -2) (myeloablative: $n = 8$; range 77-92 mg/Lxh, submyeloablative: $n = 3$; range 48-55 mg/Lxh) and serotherapy (rATG = Thymoglobuline: 5 mg/kg $n = 1$, 7.5 mg/kg $n = 3$, 10 mg/kg $n = 4$ or low-dose alemtuzumab: 0.4 mg/kg $n = 2$; 0.5 mg/kg $n = 1$). CSA and MMF were given as GVHD-prophylaxis. Preexisting complications were: colitis, pulmonary aspergillosis and bronchoectasis in 3 patients with CGD and congenital neutropenia; enteroviral CNS- and pulmonary H1N1-infections in 2 patients with SCID.

Results: Transplants comprised $n = 2$ HLA-10/10-, $n = 5$ HLA-9/10-, $n = 2$ HLA-8/10, $n = 2$ HLA-5-7/10-matched cords with 3.8-30 (med. 8) x 10 e7/NC/kg bw. After a follow-up time of 3-44 (med: 21) months, all patients survived with an overall disease-free survival rate of 100% ($n = 11/11$). $N = 2$ patients with lysosomal storage diseases (Fucosidosis and alpha-Mannosidosis) experienced primary graft failure despite myeloablative busulfan exposures and were successfully re-transplanted with a different unrelated cord or haploidentically (resulting in an event-free survival of 82%). Both had previous ATG serotherapy. No hepatic VOD or fludarabine-associated CNS-toxicity were encountered. No relevant infectious complications were observed except 2 patients with primary graft failure suffering from Candida fungemia. All preexisting infections and inflammatory complications are cleared off. The cumulative incidence rate of moderate chronic GVHD is 9% (1/11): a patient with congenital neutropenia with preexisting bronchiectasis has been suffering from moderate lung involvement after UCBT, however, has been successfully tapered off immunosuppressants. Three patients with SCID, HLH and CHH were successfully engrafted after low submyeloablative busulfan exposures, $n = 2$ had up-front low-dose alemtuzumab administration. Two patients with lysosomal storage disease other than Hurler's disease were obviously difficult to engraft even when the cumulative busulfan exposure was myeloablative demonstrating that type, dose and timing of serotherapy, HLA-matching and NC-cell content may be of additional importance. Nevertheless, myeloid donor chimerism at last follow-up is 100% in $n = 11/11$ patients.

Conclusion: High-dose fludarabine, targeted busulfan and serotherapy conditioning may help lowering the busulfan exposure in selected non-malignant diseases, i.e. SCID, CHH and HLH, transplanted with UCB with no additional need of other alkylating drugs, e.g. Thiotepa. For the UCBT of phagocyte disorders and neurometabolic diseases myeloablative busulfan exposure is recommended.
Disclosure of Interest: None declared.

P699
Haplo-identical transplantation for high-risk, refractory or recurrent pediatric acute leukemia and MDS

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Introduction: To improve the benefit of HSCT for the patients with recurrent or refractory acute leukemia, we performed HSCT following the salvage chemotherapy and evaluate the efficacy and safety profile.
Materials (or patients) and methods: A total of 27 consecutive patients with recurrent or refractory acute leukemia at our center from 2009 to 2014 are analysed. All patients were tranplanted not in the status of remission. Leukemic blasts were counted in the bone marrow range from 7% > 98%. Of them, 10 patients were transplanted from 10/10 HLA matched related donors, while 17 from haplo-identical donors. The median age of the patients is 22 (range 1-52) years. Before the conventional regimens Bucy followed by preemptive donor lymphocyte infusion, all patients received a course of salvage chemotherapy including fludarabine and cytarabine for 5 days. The GVHD prophylaxis consisted of ATG, CsA, MTX and anti-CD25. None of these patients had severe infection or organ failure before HSCT.
Results: All patients achieved successful engraftment within one month post transplant, among which 25 patients were detected complete donor chimerism and 2 mixed donor chimerism. More than half patients (85%) remain alive after a median of 22 months (range, 2 - 60). Nonrelapse mortality (NRM), and relapse incidence were 7.4% and 7.4%, respectively. Severe acute-GVHD and chronic-GVHD appeared in 2 patients, respectively. In a multivariate analysis, blast counts and the time of HSCT affected both leukemia-free survival (LFS) and NRM.
Conclusion: Following the salvage chemotherapy, HSCT appears advantages for the patients with recurrent or refractory acute leukemia without increasing NRM.
Disclosure of Interest: None declared.

P700
T- and B-cell depleted haploidentical stem cell transplantation in relapsed metastatic high-risk neuroblastoma

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Introduction: Outcome in patients with relapsed metastatic neuroblastoma is still very poor despite advances in chemotherapy and surgery. There is growing evidence for Graft-versus-tumor effects in solid tumors after haploidentical stem cell transplantation. We present an analysis of 2 prospective trials with 31 patients who received T- and B-cell depleted haploidentical stem cells
Materials (or patients) and methods: All donors were ≥2 HLA loci mismatched parents. G-CSF mobilized peripheral stem cells were T-cell depleted by using CD3-coated microbeads. B-cell depletion was done by either using CD19-coated microbeads or by in vivo depletion with antiCD20-mAb (Rituximab).
14 Patients received MIBG-therapy during 4 weeks prior to transplantation. The myeloablative conditioning consisted of fludarabine (40 mg/m^2), thiotepa (2 x 5 mg/kg) and melphalan (2x70 mg/m^2). One patient was treated without melphalan and fludarabine was replaced by clorafabine (4x50 mg/m^2) in one patient. OKT3 was given as graft rejection prophylaxis (0-1 mg/kg per day for 10 days) and was replaced in 2012 by ATG-F (30 mg/kg total dose, $n = 4$ patients). Short course Mycophenolate mofetil was given as prophylactic immune suppression, if residual T cells in the graft exceeded 2-5 x 10^4/kg BW.G-CSF was routinely started on day +4 in 20/31 patients.
Remission status before transplantation after salvage therapy was: CR ($n = 4$ patients), PR ($n = 20$ patients), non-response/progression ($n = 7$ patients). 19 patients have been transplanted in first and 6 patients in second or subsequent relapse. 6 patients never reached remission before SCT.
Results: 8/31 patients are alive after a median follow-up of 5.24 years (0.92-9.28). 5-year overall and event-free survival was 22% and 20% respectively.
Primary engraftment was observed in 28/31 (91%) patients, 3 (9%) patients rejected and were rescued with an autologeous backup. No transplant related mortality was observed.
23 Patients relapsed in the first year after transplantation. Relapse was very unlikely after 1 year.
ANC was reached after a median of 11 days (9-33). We observed a rapid increase of natural killer cells in the early posttransplant period, reaching 230 cells/µl in the mean (SD = 113) on day 21. Regeneration of T- and B-cells started around day 30 and reached 110/µl (SD = 193) and 38/µl (SD = 43) in the mean at day 90 and 292/µl (SD = 285) and 177/µl (SD = 172) at day 180.
Toxicity was tolerable. 26 patients (84%) suffered from °III-IV gastrointestinal toxicity including mucositis, nausea and diarrhoea. 3-fold elevated liver enzymes were found in 10 patients (32%). Hypoxia requiring O2-Substitution occurred in 3 patients. No cardiac, neurological, renal or dermatological °III-IV toxicities were observed. No veno-occlusive disease occurred.
Acute °I-II GvHD was observed in 21 and °III in 3 patients. No °IV aGvHD was observed. Chronic °I-II GvHD occurred in 11 patients. No °III-IV cGvHD was observed.
Conclusion: Haploidentical stem cell transplantation is feasible for treatment of patients with relapsed neuroblastoma with acceptable toxicity and morbidity. Incidence of GvH was low. However, NK cell mediated GvT effects still have to be evaluated and seem to be not sufficient in the majority of patients since 80% relapsed. Thus, additional post transplant means to increase GvT effects on the basis of the new established immune system like use of anti-GD2 mAbs, DLI and cytokine stimulation need to be urgently investigated.
Disclosure of Interest: None declared.

P701
Effects of the Exercise Programme on Childrens' Fatigue and Quality of Life Level During and After Pediatric Hematopoietic Stem Cell Transplantation

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Introduction: Previous studies have shown the benefits of exercise programme in the physical capasity and overall health status of adults who have undergone hematopoietic stem cell transplantation (HSCT). Considerably less research efforts have focused on pediatric populations receiving the same medical

treatment. The aim of this study was investigate that the effects of exercise programme on fatigue level and quality of life for pediatric HSCT.

Materials (or patients) and methods: Eleven children [girl $n=1$ (%9.1), boy $n=10$ (%90.9)], planned to HSCT at Hacettepe University Bone Marrow Transplantation Unit, were included in this study. They have different hematologic diseases, malignant or non-malignant. The evaluation of the children was performed before HSCT, at the discharge day and after one month by the same physiotherapist. Some medical records were collected: height, weight, body mass index (BMI), type of transplantation, number of months after diagnosis. The exercise programme was composed of strengthening, stretching and relaxing exercises. Exercises were performed during hospitalization and these exercises were given children as a home exercise programme after discharge. Childrens' quality of life level were assessed by PedsQL 3.0 Cancer Module, and childrens' fatigue level were assessed by Wong-Baker Faces Scale.

Results: Mean age of the children was 10.00 ± 3.94 and mean duration after their diagnosis was 59.50 ± 48.63 months. Mean BMI of the children was 16.66 ± 1.86. Allogeneic bone marrow transplantation was performed to all children and HLA matchings were 10/10. During exercise sessions it didn't occur any complication. The results of this study revealed istatistically significant increase childrens' quality of life scores reported by children ($P=0.006$). And, we found istatistically significant increases for PesQL 3.0 Cancer Module procedural anxiety and communication sub-scores between time points ($P=0.021$ and $P=0.014$, respectively). Considering PedsQL 3.0 child self-report total cores between time points, there was found significant increase of the total scores between before HSCT and during discharge ($z=-2.197$, $P=0.028$) and between before HSCT and after one month ($z=-2.666$, $P=0.008$). And, we found fatigue level decreased between time points, but it was not istatistically significant.

Conclusion: The results of this study revealed exercise programme can increase childrens' quality of life during HSCT. Also, not observed the positive effects of exercise on fatigue may be due to difficulties in the assessment of fatigue for children in these age groups. About this topic, there is need for exercise and its' effectiveness on symptoms, physical status and quality of life during HSCT for children in larger populations.

Disclosure of Interest: None declared.

P702

Impact of Clinical Phenotype of Wiskott-Aldrich Syndrome on Outcomes after Unrelated Cord Blood Transplantation: a Risk Factor Analysis

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Introduction: Wiskott-Aldrich syndrome (WAS) is a severe X-linked recessive immune deficiency disorder caused by mutations in the gene encoding for WAS protein (WASP), causing thrombocytopenia, immunodeficiency, eczema, and propensity to malignancy and autoimmune diseases. A scoring system was introduced to distinguish the phenotypes of WAS: X-linked thrombocytopenia (score <3) from classic WAS (score ≥ 3), based on the severity of the clinical phenotype.

The only curative therapy for patients (pts) with WAS is HSCT. To date, there are only few reports on outcomes after unrelated umbilical cord blood transplantation (UCBT).

Materials (or patients) and methods: We analyzed outcomes of UCBT in 90 pts with WAS, who received 1st, single, unrelated UCBT from 1996-2013. Median age at UCBT was 1.5 (0.48-14) years (yr) and median time from diagnosis to UCBT was 1 (0.06-13) yr. Clinical score was known for 79 pts: 18 (20%) had a score of 2, and 61 (80%) had a score ≥ 3. Data on mutation were available for 49/90 pts. HLA matching between UCB and recipient was: 6/6 (12%), 5/6 (60%), or 4/6 (28%). Median TNC and CD34+ doses at collection were $7x10^7$/kg and $3x10^5$/kg, respectively. The vast majority of pts ($n=82$) received busulfan based myeloablative conditioning. Most (88%) received ATG. GvHD prophylaxis was CsA-based in 93% pts, 20% received MTX.

Results: Median follow-up was 60 (3-208) months. Cumulative incidence (CI) of neutrophil and platelet recovery at 60 days (d) was 88% and 75%, respectively. In multivariate analysis (MVA), no MTX in GvHD prophylaxis ($P=0.02$) was independently associated with neutrophil engraftment and age <2 yr ($P=0.005$) with platelet engraftment.

Ten patients failed to engraft, 5 died, from which 4 within 30d after UCBT, and 5 received a subsequent HSCT, from which 4 are alive at last follow-up. No secondary graft failure was reported. CI of 100d acute GVHD (grade II-IV) was $38 \pm 5\%$ and 1-yr chronic GvHD was $16 \pm 4\%$.

The probability of 5-yr OS was $75 \pm 5\%$. Twenty-three pts died; 14 of infections (7 before d-100), 3 of GvHD and the remaining 6 from other TRM. Five-yr EFS was $70 \pm 5\%$. In MVA, age <2y at UCBT, clinical phenotype (score <3) were independently associated with better OS ($P=0.001$, $P=0.002$, respectively) and EFS ($P=0.02$ and $P=0.03$, respectively). In fact, patients with clinical score <3 had an OS of 89% and EFS of 88% compared to clinical score ≥ 3, 70% and 63%, respectively. Year of UCBT >2007 was also associated with improved OS in MVA ($P=0.03$).

Chimerism data at 100 ± 30d post-UCBT were available for 66/77 and, at last assessment, for 51/62 pts who were alive and engrafted at the specific time-point. At d-100 ± 30, 68% and 32% of pts had full and mixed donor chimerism, respectively; and at last assessment, 80% and 20%, respectively.

Patients were considered to have immune recovery if absolute number of CD4+, CD8+ and CD19+ cells, at last assessment, reached at least the lowest level of age-related normal values. In this study, 24/35 pts, with available information, achieved immune recovery. Of note, all pts who did not achieve immune recovery had received immunosuppression therapy for acute GvHD.

Conclusion: In conclusion, our study confirms that UCBT is an acceptable alternative transplant approach for children with WAS, achieving high levels of donor chimerism. Younger age at transplantation and clinical phenotype are important risk factors for better outcomes.

Disclosure of Interest: None declared.

P703
the long-term follow-up of allogeneic hematopoietic stem cell transplantation in β-thalassemia major patients: A 23-year iranian experience

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Introduction: The only curative treatment for thalassemia major is allogeneic hematopoietic stem cell transplantation (HSCT). Iran, having had 23 years of HSCT experience with more six hundred cases, not only has surpassed all the developing countries' registered records but also has achieved the status of the second rank in the world after the Pesaro Group in Italy.
Materials (or patients) and methods: In this retrospective study, 674 patients (284 Female, 390 Male) underwent a risk adopted HSCT from matched related donors in Iran. The conditioning regimen was based on risk groups and age. Myeloablative conditioning regimen was used in classes I and II patients, and class III with below 15 years of age with Busulfan and Cyclophosphamide with or without ATG. But in class III patients with the age 15 and above, reduced intensity conditioning regimen (RIC) was used with Busulfan and Fludarabine. Cyclosporine A (CSA) and short course of Methotrexate (MTX) were used for GvHD prophylaxis. According to the risk classification from January 1991 till the end of 2013, the number of patients in class I, II, and III were 162, 262, and 250 respectively.
Results: 674 patients with median age of 8 years (Range: 2 to 30 years) received HSCT. 418 patients received HSCT from PBSC, 250 from BMSC, 5 patients from both PBSC & BMSC, and 11 patients from CBSC. With a median follow-up of 49.6 months (range:1 to 247), the median time of neutrophil and platelet recovery were 15 and 22 days respectively. The 4-year DFS and OS were 77.4% (SE: 3.39%) and 82.46% (SE: 3.09%) in class I, 80.6% (SE: 2.57%) and 83.44% (SE: 2.42%) in class II, and 62.44% (SE: 3.4%) and 70.8% (SE: 3.1%) in class III (*p*-value: 0.002 and 0.021). Acute GvHD occurred in 442 (65.6 of patients (30.1% grade I, 37.2% grade II, 26.3% grade III, and 6.4% grade IV)). Acute GvHD in patients receiving BMSCT and PBSCT was 62.4% and 74.3%, respectively. Chronic GvHD occurred in 145 / 467 (31.0%) of survived patients on day + 100 and 41 (28.3%) were extensive. Chronic GvHD in BMSCT and PBSCT patients was 23.3% and 37%, respectively. At the present time, 536 (78.9%) patients are alive. Transplant-Related Mortality (TRM) whose most common causes were GvHD (42.33%) and infection (18.9%) was found to be 15.4% (95% CI: 12.7–18.3%) at one year post HSCT.
Conclusion: It was concluded that OS and DFS could give better results in thalassemia major patients who received HSCT in lower ages and classes. Furthermore, it was confirmed that the application of an appropriate GvHD prophylaxis regimen would yield better results in decreasing the mortality rate in these patients. The use of ATG in conditioning regimen can reduce the rate of rejection in all classes particularly in class III patients.
Disclosure of Interest: None declared.

P704
Haploidentical Hematopoietic Stem Cell Transplantation with Depletion of TcR αβ (+) in Children: Erciyes Pediatric BMT Center

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Introduction: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) posses an alternative option for patients without a suitable donor. Erciyes Pediatric BMT Center is the solely pediatric center for haploidentical HSCT with depletion of TcR αβ (+) in Turkey. We would like to share our pediatric experience with a follow up period of two years.
Materials (or patients) and methods: All children who underwent haploidentical HSCT in our center from December 2012 to December 2014 were included in the study. Total 23 haploidentical HSCT in 19 children (6 relapsed/refractory AML, 5 relapsed/refractory ALL, 4 SAA, 1 HLH, 1 Gricelli syndrome, 1 JMML, 1 SCID) were performed. Transplantation-related mortality (TRM) was 13%. The regimen included ATG, Fludarabine, Thiotepa, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained $> 5 \times 10^4$/kg TcR αβ (+)
Results: The median of collected CD34 cells were 20,22 (range 3,98-35,04) x 10^6/kg. The graft had a purity of 99.9% TCRαβ depletion with a median of 0,253 (range 0.003 to 1,49) x 10^5 TCRαβ cells. The median engraftment days for myeloid and platelet were 11th, 12th day of HSCT, respectively. Grade II skin GvHD was detected in 2 patients, and treated with steroids without any further complications. However grade III, and grade IV gastrointestinal GvHD were observed in two patients. Although the patients with gastrointestinal GvHD were treated with steroid, budenosid, cyclosporine, MSC; one patient did not respond and died. The mean day of discharge was 30th day of HSCT. MMF was given as GvHD prophylaxis in 8 patients and 11 patients did not receive any immune suppressive drug. The long term follow up including immunological reconstructions were performed in 13 patients. The analysis of the immune reconstitution of the patients transplanted in haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 797 (range 126-2432)/mm3; for CD4+ helper T cells 87 (range 1–419)/mm3; CD8+ T cytotoxic cells 330 (range 95-2235)/mm3 at 28th day of HSCT.
Conclusion: Our results underline that haploidentical HSCT with depletion of TcR αβ (+) can be an option in experienced center in countries without unrelated donor program, as in Turkey.
Disclosure of Interest: None declared.

P705
NK cell functional impairment after allogeneic hematopoietic stem cells transplantation is associated with reduced T-bet and Eomes expression

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Introduction: NK cells play a major role in protection against tumors and infections. They are the first lymphocyte subset to recover after allogeneic hematopoietic stem cell transplantation (alloHSCT). Impaired NK cell function after alloHSCT has been reported but underlying mechanisms are ill-defined. Eomesodermin (Eomes) and T-bet, two T-box transcription factors, have been recently reported to regulate maturation and effector functions of murine NK cells. The precise role of Eomes and T-bet in human NK cell biology and their potential impact on NK cell function after alloHSCT are still unknown.
Materials (or patients) and methods: We analyzed by flow cytometry Median Fluorescence Intensity Ratios (MFIR) of Eomes and T-bet in CD56^{bright} and CD56^{dim} NK cells from 44 healthy controls and 72 patients undergoing alloHSCT at our center. *Ex vivo* expression of perforin was analyzed in CD56^{dim} cells. In addition, we analyzed IFN-γ production in NK cells after *in vitro* culture with tumor target K652 cells. We used non-parametric Mann-Whitney test or Spearman's rank test where appropriate.
Results: T-bet levels were strongly reduced in both CD56^{bright} [MFIR 6.2 (IQR 3.0-9.3)] and CD56^{dim} NK 7.5 (3.2-13.6) cells from HSCT recipients compared with healthy controls [CD56^{bright}

19.0 (14.6-27.2), $P<0.0001$; $CD56^{dim}$ 21.8 (17.4-31.9), $P<0.0001$]. Similarly, Eomes was expressed at significantly lower levels in both $CD56^{bright}$ [6.9 (3.3-14.3)] and $CD56^{dim}$ [4.7 (2.5-8.8)] NK cells from HSCT recipients compared with cells from healthy controls [$CD56^{bright}$ 22.6 (13.5-31.5), $P<0.0001$; $CD56^{dim}$ 11.2 (6.9-16.6), $P<0.0001$]. Importantly, we found no relationship between T-bet and Eomes expression in NK cells and time from transplantation, myeloablative or reduced intensity conditioning, and transplantation with T-cell repleted or depleted grafts. Conversely, acute but not chronic GvHD was associated with significantly reduced levels of T-bet in $CD56^{bright}$ NK cells ($P=0.0386$). CMV reactivation was associated with lower levels of Eomes in $CD56^{bright}$ ($P=0.0208$) and $CD56^{dim}$ ($P=0.0129$) NK cells and reduced expression of T-bet in $CD56^{dim}$ NK cells ($P=0.0197$). As previously reported, IFN-γ production and perforin expression in NK cells from alloHSCT recipients were lower than in healthy controls. Interestingly, we found a significant correlation between reduced T-bet levels and altered perforin expression ($r=0.2624$, $P=0.0486$).

Conclusion: The expression of the T-box transcription factors Eomes and T-bet is significantly reduced in NK cells after alloHSCT. Moreover, reduction in Eomes and T-bet expression is associated with NK cell functional impairment. Our results provide a molecular explanation for the reduced NK cell function after alloHSCT previously reported. Further studies will assess the potential of Eomes and T-bet targeting strategies to restore and improve NK cell functions after alloHSCT.

Disclosure of Interest: None declared.

P706

Intra-bone cord blood injection is associated with improved platelet engraftment compared to intravenous cord blood hematopoietic stem cell transplantation both in adult and pediatric patients

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Introduction: Cord blood hematopoietic stem cell transplantation (CBHSCT) is one of the therapeutic options suitable for patients who need hematopoietic stem cell transplantation but who lack both an HLA identical siblings and an unrelated HLA matched adult stem cell donor. One of the main weaknesses of CBHSCT is a delayed neutrophil and platelet engraftment that is reported as one of the causes of the increased risk of transplant related mortality of this kind of transplantation. Some experimental clinical trials carried out both in adult and pediatric population have suggested that by direct intra-bone injection of the cord blood unit (IBCBT) it is possible to improve the engraftment rate but studies directly comparing IBCBT and intravenous CBHSCT are still missing.

Materials (or patients) and methods: Between December 2007 and April 2014, 37 patients aged from 1 to 71 years (median age 8 years, median weight 23 kg) underwent CBHSCT at our Centers: 18 had IBCBT while 19 had intravenous CBHSCT. In order to explore if direct intra-bone injection of cord blood derived hematopoietic stem cells was associated with a faster neutrophil (PMN) and platelet (PLT) recovery we retrospectively compared the cumulative incidence of PMN and PLT recovery in the two groups. In the same study population we also analyzed the impact of variables that have been previously reported as important factors in determining CBHSCT outcome, as recipient's age and weight, Total Nucleated Cell dose, $CD34^+$ cell dose, and number of HLA mismatches in the donor-recipient couple.

Results: We first evaluated in the entire study population ($n=37$) if younger age was associated with an improved PMN and PLT engraftment but surprisingly we didn't find any statistically significant difference ($P=$ 0,35 and $P=$ 0,68 respectively). We didn't highlight a correlation between PMN and PLT engraftment and recipient's weight either ($P=$ 0,2 and $P=0,8$ respectively). Considering PMN we didn't observe any difference between engraftment rate of patients treated by IBCBT and that of patients treated by intravenous CBHSCT at any time point. At day + 100, cumulative incidence of PLT recovery was 67% (CI95% 48-92) for patients undergone IBCBT versus 45% (CI 95%28-73) for patients undergone intravenous CBHSCT ($P=0,18$). Extending the follow up to more than a year cumulative incidence of PLT recovery was 72% (CI95% 54-96) for patients undergone IBCBT versus 50% (CI 95%32-77) for patients undergone intravenous CBHSCT ($P=0,03$). About PLT engraftment we found no statistically significant differences according to Total Nucleated Cells infused, CD34 + cells infused and HLA mismatches in donor-recipient couple and when we considered the association between TNC and $CD34^+$ cells separately in the IBCBT group ($n=18$) and in the intravenous CBHSCT group ($n=19$) we observed a trend toward a stronger impact of cell dose in the intravenous CBHSCT group.

Conclusion: IBCBT has been shown to be one of the most reliable solutions to overcome some of the limitations of CBHSCT, but data justifying the risks related to this procedure are still missing. Our data suggest that by intra-bone injection it is possible to overcome the problem of delayed platelet engraftment in CBHSCT even if further data are needed to confirm the results obtained.

Disclosure of Interest: None declared.

P707

Clinical and molecular features associated with acute myeloid leukemia engraftment in immunodeficient mouse models

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Introduction: Over the last decade, immunodeficient mouse models have been extensively used to assess the in vivo growth potential of human leukemia, to provide insights into its biology, and to perform preclinical validation of therapies. In the present study we characterize the clinical features and gene expression signature associated with leukemia engraftment into mice, with the ultimate aim to identify novel clinical prognostic factors and therapeutic targets.

Materials (or patients) and methods: Acute Myeloid Leukemia (AML) samples harvested at diagnosis from 26 patients were purified and infused into non-irradiated immunodeficient NOD/SCID γ-chain null (NSG) mice. Upon leukemia engraftment, assessed by multiparametric flowcytometry, mice were sacrificed and leukemic cells were isolated, characterized, and reinfused in serial recipients, in up to four serial passages. Gene expression profile was analyzed using Illumina microarray, and deregulated genes and processes were identified by pairwise LIMMA (Linear Model for Micro-Array Data)analysis, comparing primary leukemia with xenografts harvested from mice at first and at fourth serial transfer. Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA) curated databases were interrogated to identify deregulated processes.

Results: Twelve out of 26 primary samples (46%) generated AML xenografts. Engraftment into mice strongly correlated with poor patient prognosis, and in particular displayed a highly significant correlation with the patient subsequently experiencing relapse after allogeneic transplantation (OR:36, 95% CI: $P=0.007$).

Engraftment and growth kinetics of the human leukemic cells were highly consistent among littermates, and reproducible amongst different experiments. Notably, upon serial transfer AML exhibited an accelerated and more aggressive growth kinetic.
The gene expression profile of xenografts was reproducible and consistent amongst littermates. Genes deregulated in xenografts accounted for 9.1% of the transcript assessed (4.6% upregulated, 4.5% downregulated), with substantial overlap in the genes and processes deregulated in each of the studied cases. GO and GSEA demonstrated the selective deregulation of genes involved in cell proliferation (DLGAP5, CENPF, CDC20, AURKA, AURKAPS1), myeloid differentiation (AZU1, RNASE3, CTSG, ELANE, DEFA1B, DEFA3, DEFA1) and antigen processing and presentation (ICAM1, IFI30, FCGRT, CD1A, CD1B, CD1D, CD1E, HLA-DR, HLA-DP, HLA-DQ). Notably, in one of the xenografts we could clearly identify an expression signature reportedly associated with cancer stem cells[1].
Conclusion: In our study, we characterized the expression signature acquired by leukemic cells engrafted in one of the most commonly used immunodeficient mouse strain, and identified genes related to an aggressive behavior in this model. Translation of these results into the clinical setting might provide novel markers of leukemia aggressiveness and rationales for targeted therapies.
References: [1]Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat Med. 2011;17:1086-93.
Disclosure of Interest: G. Oliveira: None declared, C. Caserta: None declared, G. Bucci: None declared, C. Toffalori: None declared, B. Camisa: None declared, L. Crucitti: None declared, L. Zito: None declared, M. G. Carrabba: None declared, M. Bernardi: None declared, A. Bondanza: None declared, C. Bonini Conflict with: scientific consultant of MolMed S.p.A., F. Ciceri: None declared, L. Vago: None declared.

P708
Alpha/Beta T- Cell Depleted Allogeneic Stem Cell Transplantation From Matched Related And Unrelated Donor Grafts In Patients With High Risk Hematological Malignancies

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Introduction: The outcome of allo-SCT in patients with poor risk hematological malignancies is still hampered by GVHD and relapse. The innate immune system has been reported to contribute to tumor control, with lower incidence of GVHD. Specific depletion of αβ T- cells – key players in the development of GVHD – will render NK cells and γδ T cells within the allograft. Recently reported results have shown the great promise of this approach in haploindentical transplantations. Within this study, we aim to extend αβT- cell depleted allo-SCT to patients with a MRD or MUD.
Materials (or patients) and methods: Patients with high risk hematological malignancies were included in this phase I study. Either HLA matched siblings (MRD) or HLA matched (9 or 10/10) unrelated donors (MUD) were eligible. αβT-cell reduction was performed by negative selection with anti-αβTCR antibodies in combination with magnetic microbeads, using the automated CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany). The maximal contamination with abT-cells for all dose levels was $5x10^5$/kg. The conditioning regimen consisted of: ATG (Genzyme®) 4 or 6 mg/m^2 + fludarabine 120 mg/m^2 + busilvex AUC = 90 followed by αβT-cell depleted grafts from matched related or unrelated donors. No additional immune suppression was given after allo-SCT.
Results: Products for 15 patients have been successfully processed and used for αβT-cell depleted allo-SCT between 2013 and 2014. A ~4 log depletion of αβT-cells has been observed in the product with a recovery of ~75% of CD34$^+$ cells. The combination of ATG/fludarabine/busilvex was well tolerated with a hematological recovery within 3 weeks. Primary engraftment (chimerism > 95%) was observed in all patients. Immune reconstitution primarily consisted of innate cells (NK cells and gd T cells) the first 6 months post transplantation. In addition, no increase in CMV or EBV reactivations has been observed so far under the profound "innate control". Up to date, none of the patients developed aGVHD > grade II.
Conclusion: ATG Busulfan Fludarabine is a low toxicity platform for αβTCR-depleted transplantations, resulting in a swift reconstitution of innate cells (NK cells and γδ T cells) the first 6 months post transplantation. This transplantation strategy can serve as a tool for future immunological interventions such as a pre-emptive DLI or transfer of genetically modified T cells.
Disclosure of Interest: None declared.

P709
Abstract Withdrawn

P710
Comparison of Cellular Immune Reconstitution for two different method of Haploidentical Hematopoietic Stem Cell Transplantation in children (Post Transplantation Cyclophosphamide versus Negative Depletion of TcRαβ(+)); Preliminary results

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Introduction: The depletion of T cell receptor αβ$^+$ (TcRαβ(+)) T lymphocytes and pharmacological lymphocyte depletion with post-transplant cyclophosphamide (PTCY) are the recent approaches for haploidentical HSCT. Immune reconstitution after both approaches for haploidentical HSCT has not been studied. Therefore we analyzed the immune reconstitution and clinical outcome in 23 children receiving haploidentical HSCT after negative depletion of TcRαβ(+) T lymphocytes and PTCY.
Materials (or patients) and methods: Between 2011 and 2014, 23 patients (6 female, 17 male) were enrolled in the study. 13 of them received TcRαβ(+) haploidentical HSCT at the Erciyes University, Kayseri; and 10 patients received haploidentical HSCT with PTCY at the Bahcesehir University, Medical Park Hospital, Antalya. Reconstitution of lymphocyte subsets (CD3, CD4, CD8) was monitored monthly by flow analysis until six months, followed by an assessment every 3 months.
Results: The median age of 13 children was 8 years (range: 2-14 years) in the TcRαβ(+) haploidentical HSCT group. Five patients had ALL, 4 had AML and 4 had severe aplastic anemia. Nine mothers and 4 fathers were chosen as donor. The median number of transplanted CD34 stem cells was 19.7 x 10(6)/kg (range12.2-35.0). The median time to myeloid engraftment was 11 days (range 10-24) and platelet recovery was 12 days (10-31). Mycophenolate mofetil was given as graft-versus-host disease (GVHD) prophylaxis in 8 patients and 5 patient did not receive any posttransplantation pharmacologic prophylaxis for GVHD in the TcRαβ(+) haploidentical HSCT group. On the otherhand, the median age of 10 children was 11.5 years (range:7-17 years) in the haploidentical HSCT with PTCY group. Seven patients had ALL, 1 had severe aplastic anemia, 1 had relapsed non Hodgkin Lymphoma and 1 had adrenoleukodystrophy. Five mothers, 4 fathers and 1 sibling was chosen as

donors. The median number of transplanted CD34 stem cells was 6.4 x 10(6)/kg (range1.5-16.0). The median time to myeloid engraftment was 16 days (range 14-20) and platelet recovery was 16.5 days (11-29). Cyclophosphamide, cyclosporine, mycophenolate mofetil were given as GVHD prophylaxis in all patients at the PTCY group.
All patients in both groups engrafted but two patients in both groups relapsed in the later period. Two patients experienced acute GVHD and no patient developed chronic GVHD in both groups. The analysis of the immune reconstitution of the patients transplanted in TcR$\alpha\beta(+)$ haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 797 (range 126-2432)/mm3; for CD4+ helper T cells 87 (range 1–419)/mm3; CD8+ T cytotoxic cells 330 (range 95-2235)/mm3 at 28th day of HSCT. Second months the PTCY group showed a rapid immune reconstitution in all lymphocyte subsets. Than TcR$\alpha\beta(+)$ HSCT group showed a rapid immune reconstitution to the one year follow up period. But differences are not statistically significant.
Conclusion: Our data suggest that both novel graft manipulation strategies are safe and effective for haploidentical HSCT. Immune reconstitution is rapid in the TcR$\alpha\beta(+)$ haploidentical HSCT group but all lymphocyte subsets results are much closed to each other.
Disclosure of Interest: None declared.

P711
Radiation dose and cell dose determine graft survival in a canine hematopoietic stem cell transplantation model

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Introduction: The canine hematopoietic stem cell transplantation (HSCT) model has become accepted during the last decades as a good preclinical model for the development of new transplantation strategies. Information on factors associated with outcome after allogeneic HSCT are a prerequisite for designing new risk adapted transplantation protocols. Here we report on a retrospective analysis aimed at identification of risk factors for allograft rejection in the canine HSCT model.
Materials (or patients) and methods: A total of 74 dog leukocyte antigen-identical sibling HSCT were performed since 2003 on 10 different protocols. Three HSCT recipients were not evaluable and were therefore not included into this analysis. The median age of the recipient was 17 months (min7-max32) and the median weight was 13.8 kg (min9.8-max20.0) at the time of transplant. 38 recipients were male (54%). Conditioning consisted of 1 Gy ($n=20$), 2 Gy ($n=37$) and 4.5 Gy ($n=14$) total body irradiation (TBI) based regimen. CSA alone or different combinations of CSA, MMF and RAD001 were used for immunosuppression. A median cell dose of 3.6 total nucleated cells (TNC)/kg (range 1.0-11.8) was infused. Follow-up was restricted to 26 weeks after HSCT in 8 protocols and to 16 weeks in 2 protocols. Uni- and subsequent multivariate Cox analyses were used to assess the influence of age, weight, TBI dose, gender of donor/recipient, type of immunosuppression and cell dose (TNC, CD34+) on allograft rejection.
Results: Initial engraftment occurred in all dogs. Animals that received a 1 Gy conditioning eventually rejected their graft after median 10 weeks (min7-max16). After 2 Gy TBI 15/37 dogs (41%) achieved stable long-term engraftment (>26 weeks), 5 dogs died before end of the study. At a TBI dose of 4.5 Gy 13/14 dogs (93%) showed stable donor chimerism during study period (16 weeks) and 1 dog died. Univariate analyses revealed TBI dose, age and weight of the recipient, gender and TNC dose as factors influencing long-term engraftment ($P<0.2$ each). In multivariate analysis 2 factors were identified as significant independent risk factors for graft rejection: low TBI dose and TNC cell count ($P<0.05$ each). PBMC chimerism >30% and granulocyte chimerism >70% 4 weeks after HSCT were identified as independent predictors for stable engraftment ($P<0.001$ each).
Conclusion: In summary, these data support that even in low dose TBI based regimen irradiation dose is important for engraftment. In addition, TNC dose were identified as risk factor of graft survival. Level of blood chimerism at 4 weeks post HSCT was predictive for long-term engraftment in the canine HSCT model.
Disclosure of Interest: None declared.

P712
Safety and efficacy of direct intrabone transplantation of peripheral blood hematopoietic stem cells from HLA-matched sibling donors in patients with myeloid and lymphoid malignancies – results of a pilot phase I/II study

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Introduction: Based on the animal studies, only 10-15% of intravenously transplanted stem cells migrate to hematopoietic sites while the rest is lost in other organs. Intrabone (IB) route of administration may readdress the fate of stem cells increasing their homing capacity. Results of IB allogenic cord-blood cells transplantation (tx) in humans confirm that it may be associated with less probability of graft failure and may reduce risk of GVHD and malignancy relapse. In the current phase I/II study we investigate IB route of allogeneic peripheral blood stem cell (PBSC) tx.
Materials (or patients) and methods: The study is registered to ClinicalTrials.gov (identifier: NCT01728389). The primary end-point is the probability of neutrophil and platelet engraftment. Between X.2012 and VI.2014 seven out of 10 planned patients, aged 42 years (25-53) were enrolled. All of them suffered from high risk malignancies (2 - AML adverse genetic risk group in CR1; 2 - ALL Ph(+) in CR1 without molecular remission; 1 - ALL common in CR1, MRD(+) after induction; 1 – T-cell lymphoblastic lymphoma in PR2; 1 – CLL, 17p(-) in CR3). The conditioning regimen was myeloablative, based on TBI, and immunosuppressive regimen was CsA and short course of Mtx in all cases. PBSC harvest was performed from sibling donors using Spectra-Optia Apheresis System (TherumoBCT Inc, Lakewood, CO, USA). Part of harvested stem cells (at least 4 x 10^6/kg) was cryopreserved as a back-up. Part intended for tx (median volume 180 ml (112-250), median CD34+ content 5.1 (4.9-6.5) x 10^6/kg) was processed. To reduce the volume of cell suspension, the apheresis product was centrifuged in transfer bags (15 min. 1000 RCF, RT). The supernatant was removed and the pelleted cells were resuspended in 30-40 ml of donor plasma and then divided into syringes (6-7 ml each). Median product volume after processing was 36 ml (26-50), 6 (5-8) syringes. Tx was performed under short-time intravenous sedation. Patient was positioned in the flank posture. A needle was inserted into the posterior-superior iliac crest bone-marrow cavity. Subsequently, 6-7 ml of processed stem cell suspension was slowly infused. The needle was then rinsed with 1 ml of saline. This maneuver was then repeated for all the remaining stem cell portions at a distance of about 3-4 cm from the previous injection site, across the both iliac crests.
Results: There were no early and late side effects of IB injection of stem cells noticed. All patients engrafted. A median time to neutrophil recovery >0,5 G/l was 13 days (10-15), platelets >20 G/l – 13 days (1-23) and platelets >50 G/l - 14 (13-23). G-CSF was used to hasten neutrophil recovery in 3 pts. Full donor chimerism was achieved in 5 (71%) of pts on day 30 and in all pts on day 100. Acute GVHD occurred in 3 cases (2 – I; 1 – III grade), whereas chronic in 3 (2 – extensive, 1 – limited). 3 pts relapsed and 2 of them died due to relapse. One patient died due to refractory Clostridium difficile infection and acute GVHD III (skin 2, gut 2). 4 patients remain alive with the median follow-up of 16 months.

Conclusion: IB route of PBSC tx if feasible, safe and allows to achieve fast engraftment. Further investigation is warranted to check biological aspects of this attempt and also its impact on relapse and GVHD risk.
Disclosure of Interest: None declared.

Acute leukaemia II

P713
Prognostic impact of the European LeukemiaNet standardized reporting system in acute myeloid leukemia patients receiving hematopoietic stem cell transplantation after non-myeloablative conditioning

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Introduction: The prognosis of most acute myeloid leukemia (AML) patients (pts) is still poor. Non-myeloablative conditioning (NMA), with a therapeutic approach mainly bases on a Graft-versus-Leukemia effect, opened the possibility of hematopoietic stem cell transplantation (HCT) for older or medically unfit pts. The European LeukemiaNet (ELN) suggested a standardized reporting system for AML pts, but the prognostic significance of this system for pts receiving NMA-HCT is unknown.
Materials (or patients) and methods: We analyzed 216 pts that were treated between April 1999 & December 2012 at the University Hospital Leipzig. Median age was 64 years (y, range 36-76y). We included pts with *de novo* ($n=126$; 58.3%), secondary ($n=70$; 32.4%) or therapy-related ($n=20$; 9.3%) AML. All received NMA-HCT: either fludarabine (30 mg/m^2 at day -4 to -2) & 2 Gy total body irradiation (TBI; $n=209$; 96.8%) or fludarabine (30 mg/m^2 at day -4 to -2) & 3 Gy TBI ($n=1$; 0.5%) or 2 Gy TBI ($n=6$; 2.8%). Donors were human leucocyte antigen (HLA)-matched related ($n=35$; 16.2%) or HLA-matched ($n=120$; 55.6%) or mismatched ($>=1$ antigen; $n=61$; 28.2%) unrelated. Cytogenetics & presence of *FLT3*-ITD & *NPM1* & *CEBPA* mutational status at diagnosis were assessed centrally.
Results: For the entire cohort the median overalls survival (OS) after transplantation was 2.02y, with a 2-y-OS of 50.1% [95% Confidence Interval (CI) 43.5-57.7%].
23.4% of pts were *NPM1* & 15.6% *CEBPA* mutated (mut), while 16.8% had a *FLT3*-ITD. 88 (40.7%) pts had a normal karyotype. When pts were classified according to the ELN standardized reporting system we found 19.4% with favorable (fav; $n=42$; including 16.7% [$n=7$] with a core-binding factor AML, 38.1% [$n=16$] *CEBPA* mut & 45.2% [$n=19$] *NPM1* mut/no *FLT3*-ITD with normal karyotype), 24.5% with intermediate-I (int-I; $n=53$; including 18.9% [$n=10$] *NPM1* mut/*FLT3*-ITD, 11.3% [$n=6$], *NPM1* wild type [wt]/*FLT3*-ITD, 69.8% [$n=37$] *NPM1* wt/no *FLT3*-ITD with normal karyotype), 23.1% with intermediate-II (int-II; $n=50$; including 2.0% [$n=1$] with t(9;11)]) & 32.9% with adverse (adv; $n=71$; including 77.5% [$n=55$] with complex karyotype; 7.0% [$n=5$] with monosomy 7; 12.7% [$n=9$] with monosomy 5 or loss of 5q; 2.8% [$n=2$] with inv(3)) genetic risk. We analyzed the CIR according to ELN groups and found a statistical significant separation for the whole cohort ($P=.037$; Figure1A). This was also found by trend for OS ($P=.104$; Figure1B). Analyzing the OS among each group, only fav was performing significantly better than int-II ($P=.054$) & adv ($P=.022$).
Conclusion: To our knowledge, we are the first to demonstrate the prognostic significance of the ELN reporting system for AML pts receiving NMA-HCT. For younger (≤ 60y) pts receiving high-dose chemotherapy for consolidation, several studies revealed that while fav performed best & adv worst, int-II had better outcome than int-I & that in older pts int-I & int-II had comparable outcomes. In our set, we did not find that separation and only fav performed better than int-II or adv pts. However, it seems like the prognostic impact between the groups diminishes. Thus, especially older or unfit pts with int-I or adv cytogenetic risk might benefit from NMA-HCT.
Disclosure of Interest: None declared.

P714
Autografting in AML: CD96 antibody TH-111 removes leukemic stem cells from grafts

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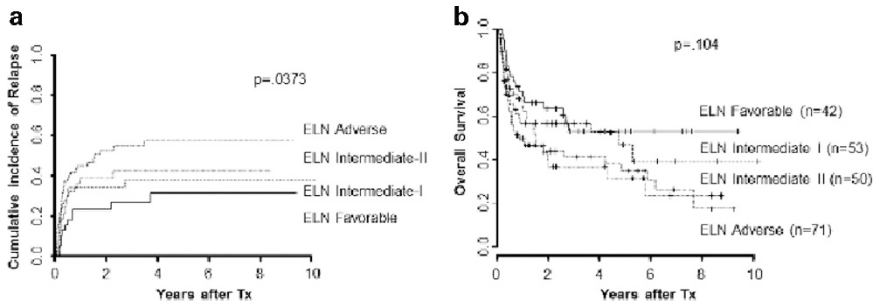
Introduction: The high relapse rate observed after autologous stem cell transplantation (ASCT) in patients with acute myelogenous leukemia (AML) limits this therapy strategy. Therefore, the goal is to remove residual leukemic stem cells (LSC) at least from the graft. The monoclonal antibody TH-111, raised in our laboratory, targets the CD96 antigen characteristically expressed on AML-LSC. Here, a strategy is implemented to remove CD96-positive LSC from autologous grafts by magnetic cell sorting (MACS). In addition, antibody engineering improves antibody dependent cell-mediated cytotoxicity (ADCC) against residual AML-LSC for therapeutic targeting *in vivo*.
Materials (or patients) and methods: Following biotinylation the CD96 antibody TH-111 was used for MACS based depletion of AML-LSC cells. Cell viability and differentiation capacity of healthy hematopoietic progenitor cells (HPC) after separation and the depletion efficiency were analyzed by immunostaining and flow cytometry as well as in colony forming assays. Recombinant DNA technologies were used to generate affinity maturated and ADCC-optimized CD96 antibodies. SDS page and flow cytometry allowed to characterize the recombinant proteins for purity and specific binding.

[P713]



Antibody-mediated effector functions were analyzed in ^{51}Cr-release assays.
Results: The efficiency of antibody mediated LSC purging was determined by spiking a graft with AML cells. Using biotinylated CD96 antibody TH-111 and anti-biotin-microbeads, targeted cells could be depleted approx. 1000-fold with MACS technology. Viability of healthy HPC as well as their potential to proliferate and differentiate was not affected. Importantly, primary CD96-positive AML-LSC could be efficiently eliminated from the bone marrow aspirate of an AML patient. A chimeric antibody containing affinity maturated variable regions in combination with an ADCC optimized human IgG_1 Fc was generated. In contrast to an Fc knock-out variant, this construct efficiently recruited NK cells and lysed CD96-positive AML-LSC.
Conclusion: Using CD96 as marker for AML-LSC, column based purging technology eliminates LSC from mixed cell populations. Moreover, a chimeric affinity maturated and Fc-optimized CD96 antibody was able to recruit NK cells for lysis of AML-LSC. Therefore, graft-engineering strategies focusing on CD96 are feasible and avoid contamination of the autograft with AML-LSC. This strategy may help to revitalize ASCT in AML. In addition, the design of CD96 antibodies opens up therapeutic avenues in eliminating residual disease also in the allogeneic setting.
Disclosure of Interest: None declared.

P715
The place of allogeneic hematopoietic stem cell transplantation in the therapeutic management of de novo and secondary acute myeloid leukemia

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Introduction: We evaluated the therapeutic management of AML patients followed in our center between July 2007 and September 2013, after stratification on age, prognosis, with or without allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Materials (or patients) and methods: A total of 572 consecutive patients were included; 311 (54%) males and 261 females with a median age of 63 years (range: 20-92), 406 (71%) were *de novo* and 166 (29%) secondary AML. Cytogenetic and molecular biology data were collected for all patients and prognosis was differentiated according to the European LeukemiaNet classification (Blood 2010). Accordingly, 335 (59%) patients were unfavorable, 83 (15%) favorable, 48 (8%) intermediate I and 106 (18%) were intermediate II. We divided the population into "young" population with age $\leq$65 years ($N=318$, 56%) and "old" with age $>$65 years ($N=254$, 44%). In the young population, there was 4 groups, group 1: favorable/intermediate1 prognosis patients who received intensive chemotherapy within or according to the Acute Leukemia French Association (ALFA) protocols ($N=105$, median age = 47 years); group 2: intermediate2 /unfavorable (poor) prognosis patients who received intensive chemotherapy within or according to ALFA protocols followed by allo-HSCT ($N=126$, median age = 50 years), group 3: patients with poor prognosis who received only intensive chemotherapy without allo-HSCT ($N=69$, median age = 57 years), and group 4: patients with poor prognosis who could not be treated due to early death ($N=18$). In the old population we distinguished, group 1: patients with good prognosis who received moderate intensity chemotherapy within or according to ALFA protocols ($N=25$, median age = 73 years); group 2: patients with poor prognosis who received azacitidine ($N=25$, median age = 76 years), group 3: patients with poor prognosis who received moderate intensity chemotherapy ($N=89$, median age = 76 years), group 4: patients with poor prognosis who received low dose Ara-C ($N=28$, median age = 76 years), group 5: patients with poor prognosis who received other treatment ($N=38$, median age = 77 years) and finally group 6: patients with poor prognosis considered as palliative or who did not receive any treatment ($N=49$, median age = 77 years).
Results: After a median follow-up of 34 months (range: 4-77) for surviving patients, the 2-years probability of overall survival (OS) in the "young" population for groups 1,2,3 and 4 was 84%, 56%, 31% and 0% respectively; and in the "old" group it was 71%, 37%, 31%, 5%, 21% and 0% for groups 1,2,3,4,5 and 6 respectively ($P<0.001$). In the "old" population, we showed that poor prognosis patients receiving azacytidine had a significantly better OS compared to those receiving low dose Ara-C ($P=0.015$). Interestingly, in the secondary AML population, the 2 years OS for fit patients who received allo-HSCT was 55% (range: 47-63) and it was 18% (range: 14-22) for those who received induction or palliative treatment without allo-HSCT with a median OS of 33 and 7 months respectively.
Conclusion: AML patients with good prognosis could achieve very good survival rates after intensive chemotherapy; allo-HSCT after induction chemotherapy remains the best therapeutic option for fit patients with poor prognosis as well as patients with secondary AML while the same fit patients with no donor or not transplanted have significantly lower survival.
Disclosure of Interest: None declared.

P716
Haploidentical second allogeneic hematopoietic stem cell transplantation for the treatment of acute leukemia relapse after first allo-HSCT: An updated retrospective registry analysis of 68 patients on behalf of the German Cooperative Transplant Group

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Introduction: 2nd allo-HSCT frequently is the treatment of acute leukemia (AL) relapsing after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Donor change to a different HLA-identical donor has not resulted in significantly different outcomes when compared to choosing the initial HLA-identical donor (Christopeit et al., JCO 2013). Feasibilty of using cells from a haploidentical donor at second allo-HSCT has been shown (Tischer et al., BMT 2014).
Materials (or patients) and methods: We performed a retrospective analysis among 68 consecutive patients (female $n=32$, male $n=36$; AML $n=55$, ALL $n=13$) from 12 German centers to interrogate the role for 2nd haploidentical allo-HSCT for AL relapsing after 1st allo-HSCT. Median age was 38.5 years (range, 16-64). Grafts at 1st allo-HSCT were from matched related (32%), matched unrelated (35%), mismatch unrelated (16%), haploidentical donors (6%), and other donors, including cord blood (7%). Median duration of complete remission (CR) after 1st allo-HSCT was 269 days (range, 18-1633). All patients received cytoreductive chemotherapy as a treatment for relapse. CR was reached at start of conditioning for

haploidentical 2nd allo-HSCT in 27%. 69% showed active disease. Disease stage was not evaluated in 4%. Myeloablative/ reduced conditioning for 2nd HSCT was chosen in 14%/ 86% To overcome the HLA barrier, 24 patients (35%) received ex vivo T-cell depletion (TCD), following either CD3/CD19 negative or CD34 positive selection. Four patients received in vivo TCD only, 2 received no TCD at all, and 38 patients (56%) received high-dose cyclophosphamide post-transplant according to the Baltimore protocol.

Results: Neutrophil engraftment was achieved after a median of 16 days (range, 8-27). 52 patients (77%) achieved CR after 2nd haploidentical allo-HSCT. Out of these patients, 28 (41%) relapsed after 2nd haploidentical allo-HSCT. After a median follow-up of 270 days, 50 patents had died, 23 from leukemia, and 27 from treatment-related causes. Cumulative incidences of leukemia-associated death/ treatment-related death were 30 ± 7%/ 48 ± 7% at 1 year, and 44 ± 7%/ 54 ± 7% at 2 years. Kaplan-Meier estimated overall survival at 1 and 2 years from haploidentical second HSCT was 38 ± 6% and 19 ± 6%.

Conclusion: Haploidentical 2nd allo-HSCT is a promising approach to the treatment of AL relapse after 1st allo-HSCT. OS rates comparable to alternative treatments can be observed. Different strategies to overcome the HLA barrier seem feasible.

This retrospective study was registered as NCT01997918 at clinicaltrials.gov.

Maximilian Christopeit and Johanna Tischer as well as Wolfgang Bethge and Christoph Schmid contributed equally to this work.

Disclosure of Interest: None declared.

P717

Do persistent blasts on a day 14 bone marrow influence survival in patients undergoing allogeneic HSCT in CR1

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Introduction: The prognostic impact of a bone marrow done 7-10 days after completion of induction chemotherapy for AML is debatable. It is not certain whether in patients undergoing allogeneic transplantation (Allo) in CR1, the presence of disease on day 14 bone marrow renders them at a higher risk of post transplant relapse or inferior survival.

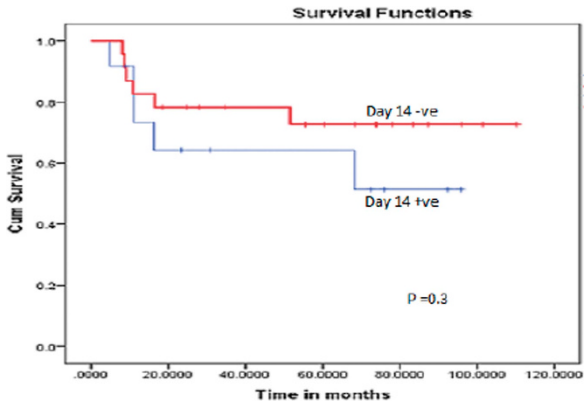
Materials (or patients) and methods: We identified 35 adolescent and young adult patients on our prospective AML database who had received induction chemotherapy with either ICE (idarubicin, cytarabine, etoposide) or standard 3 + 7 and then had an Allo in CR1. All patients had a day 14 bone marrow. The standard protocol involved giving a second cycle of re-induction with fludarabine/high dose Ara-C or mini-ICE. Patients in CR had either Allo from HLA matched sibling ($n = 34$) or from matched unrelated donor($n = 1$).

Results:

Table 1. Patient Characteristics

	D14+	*D14-*	P
N =	12	23	
Male%	7 /12 (58%)	15/23 (65%)	NS
Age, median (Range)	27.5 (14-54)	29 (16-52)	NS
WBC (Median (range)	13.8 (0.8-93)	5.7(1-52)	NS
Cytogenetic			
Good	1 /12 (8.3%)	4 / 23 (17%)	NS
Int	8 /12 (66.7%)	12/23 (52%)	NS
Adverse	3 /12 (25%)	7/23 (30%)	NS

There were no significant differences between the two groups in terms of age, sex, mean WBC or cytogenetic risk group. Among the 12 patients with day 14 marrow + ve, 11 patients had a 2nd cycle of induction before day + 21, while one patient had re-induction due to persistent blasts on day 28.



Nine pts had 2 courses of induction to achieve CR,while 3 patients had 3 induction courses. All patients with day 14 marrow –ve achieved CR with one induction course. The overall survival for the whole cohort was 68%; patients with day 14 + ve marrow had OS 58% vs 73% OS for patients with day 14 –ve marrow (P 0.3). The EFS between the 2 groups 65% vs 58% for day 14 -ve and day 14 + ve respectively (P 0.6).

Conclusion: In this cohort of young adult patients who underwent an Allo in CR1, the presence of > 5% blasts on a day 14 bone marrow biopsy did not appear to have an impact on OS or EFS, where patients are routinely given a 2nd cycle of induction for persistent disease. This data will need to be validated in a larger cohort of patients, ideally in a prospective setting.

Disclosure of Interest: None declared.

P718

Extramedullary Relapse of Acute Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT): A Single Center Experience

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Introduction: Extramedullary relapse (EMR) in leukemic patients after allo-HSCT is poorly understood and there is a few data about EMR in the literature. In this retrospective study we aimed to evaluate characteristics and outcomes of adult acute leukemia patients who had EMR after allo-HSCT.

Materials (or patients) and methods: We assessed 493 acute leukemia patients who underwent allo-HSCT between 2006 and 2014 in our transplantation unit. Of these 40 patients (29 M; 11 F) experienced EMR at the median 14 months (0.27-87.5 months) after the transplantation. In 12 patients EMR was associated with haematological relapse at the same time. We used Chi-square and Mann-Whitney U test to analyse the variance. Kaplan-Meier method was used for survival estimates.

Results: Median age was 29 years (18-55 ys). There were 26 (65%) patients with AML, 12 (30%) with ALL and 2 (5%) with biphenotypic leukemia. Cytogeneic features at pre-transplantation were: 13 high risk (32.5%), 4 good risk (10%), 3 normal karyotype (7.5%) and 19 unknown. In our cohort, only three patients had a history of extramedullary involvement prior to transplantation. Pre-transplant disease stata were: 18 patients in the 1st complete remission (CR), 11 in 2nd or 3rd CR, and 11 had active disease. Most of the patients had an HLA matched related or unrelated donor and 1 haploidentic donor. All patients received a myeloablative conditioning regimen. Total body irradiation as a part of the conditioning

regimen was used in 11 patients. The EMR localizations were central nervous system in 13 (32.5%) patients, 13 (32.5%) skin and/or soft tissue, 10 (25%) bone, 2 (5%) testicle, 1 pleura and pericardium (2.5%), and 1 visceral organ (2.5%). All patients received systemic chemotherapy alone or with donor lymphocyte infusion ($n = 15$). The response was obtained in only 13 patients. The possibility of one-year survival was $20.0 \pm 6.3\%$ from the date of relapse. When the survivals were compared according to leukemic subtype (AML vs ALL), pre-transplantation TBI use, disease status at transplantation and the response to salvage treatment, only factors that had impact on survival after EMR were pre-transplantation status and the response to salvage treatment. (1^{st} CR: $38.9 \pm 11.5\%$ vs $\geq 2^{nd}$ CR $9.1 \pm 8.7\%$ vs active disease 0%, $P = 0.007$; response (+) $38.5 \pm 13.5\%$ vs response(-) $11.1 \pm 6.0\%$, $P = 0.003$)].

Conclusion: In conclusion, our data shows that the response to salvage therapy and survival among patients with EMR was poor.

Disclosure of Interest: None declared.

P719

Acute myeloid leukemia with good and intermediate prognosis karyotype: hematopoietic stem cell transplantation or conventional chemotherapy as post 1st remission treatment? A 15 year-single center experience

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Introduction: Aggressive induction treatment with cyrarabine & anthacycline can induce complete remission (CR1) in 50-80% of adults with *de-novo* acute myeloid leukemia (AML). However, the optimal postremission therapy, especially for patients with other than poor risk karyotype, still remains an issue of debate.

Materials (or patients) and methods: We retrospectively evaluated the outcome of 118 patients (52 female/66 male) with *de-novo* AML (non M3) with a median age of ≤ 60 years and a non poor risk karyotype, who achieved CR1 after 2-5 (median 3) cycles of induction-consolidation therapy and received either hematopoietic stem cell transplantation [allogeneic (alloHSCT) / autologous (autoHSCT)] or conventional chemotherapy (CC) as post 1^{st} remission treatment. Thirty patients had good risk [t(8;21):17, inv16:13] and 88 intermediate risk (68:normal) karyotype. As postremission therapy 35 patients underwent HSCT (allo:21, auto:14, group I) while 83 received CC (group II). There were no statistical differences between groups in terms of age, sex and cycles of treatment, although in group II higher percentage of patients 26/83(30%) had good prognosis karyotype.

Results: Relapse occurred in 10 patients (3/21 post alloHSCT & 7/14 post autoHSCT) from group I and in 45 pts from group II, resulting in a probability of relapse 30% and 56% ($P < 0.01$), respectively. All relapsed patients post alloHSCT succumbed to their primary disease whereas 5/7 (70%) patients who relapsed post autoHSCT and achieved CR2, underwent 2^{nd} alloHSCT with 4 of them being in long lasting remission. CR2 was achieved by 27 patients of those who relapsed in group II (60%), however, alloSCT was feasible in 16 (35%) of them, mainly due to severe infections and the short-term remission duration. Ultimately, from the patients who relapsed, only 11(25%) attained long-term disease free survival (DFS), 9 post alloSCT and 2 with good prognosis karyotype after CC. The 15-ys overall survival (OS) for groups I and II were 75% vs. 52%, respectively ($P = 0{,}016$). Remarkably, in our study, the overall treatment related mortality (including post 1^{st} remission, salvage and post 2^{nd} remission treatment, O-TRM) for both groups was similar (10%). All but one, t8:21 και inv16 patients, who did not receive SCT as post CR1 treatment and subsequently relapsed, achieved CR2, reaching a long-term DFS 60% and 100%, respectively. We additionally evaluated the role of autoSCT vs CC as post 1^{st} remission treatment, separately for the group of AML patients with intermediate risk karyotype. The 15-ys OS was 81% for autografted pts vs. 40% for those treated only with CC ($P = 0.006$). It is noteworthy that, in patients who received autograft as post 1^{st} remission treatment, the O-TRM was 0% as compared to 13% in those who received CC as post 1^{st} remission treatment.

Conclusion: Taking into account the limitations of the retrospective analysis, our data show that in the absence of molecular prognostic markers, HSCT is a safe and the most efficacious post 1^{st} remission treatment for patients with AML of intermediate risk karyotype, significantly improving their outcome. AutoSCT represents a reliable and non toxic postremission treatment when a suitable donor is lacking. For subjects with a good prognosis karyotype, HSCT (allo or auto) as post 1^{st} remission treatment is not indicated.

Disclosure of Interest: None declared.

P720

Role of allogeneic stem cell transplantation (HCT) for FLT3/ITD acute myeloid leukemia: a single institution experience

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Introduction: Allogeneic hematopoietic cell transplantation (HCT) represents a postremission therapy that is effective at reducing the risk of relapse for many cases of poor-risk AML. The FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) molecular mutations typically confers a poor prognosis to patients with acute myelogenous leukemia (AML). In hopes of overcoming this poor prognostic factor our institution has adopted a policy of pursuing allogeneic transplantation, including the use of unrelated or alternative donors, for FLT3/ITD AML patients, as soon as possible after reaching the best response induced by chemotherapy with or without FLT3-ITD inhibitors.

Materials (or patients) and methods: from february 2009 to october 2014, 15 patients (5 males) with AML FLT3/ITD+ received an HCT from HLA identical sibling ($n = 10$), or matched unrelated donor (MUD) ($n = 2$), or HLA haploidentical donor ($n = 3$). At diagnosis all patients had a normal karyotype. Therapy at diagnosis was not uniform. No patient had received a previous transplant. The median age at transplant was 50 yr (range, 21-64). Five patients received 2 or 3 previous chemotherapy lines. At transplant 10 patients were in CR1, 4 patients in CR2 and 1 patient was in PR. All but one patient received a myeloablative conditioning based on intravenous busulfan combined with cyclophosphamide (2 patients) or fludarabine (12 patients) and thiotepa (8 patients). The stem-cell source was bone marrow in 10 patients and peripheral blood in 5 patients. Graft-versus-host disease (GvHD) prophylaxis was cyclosporine combined with short course methotrexate for all patients, but three patients transplanted with unmanipulated bone marrow stem cells from haploidentical donor received also mycophenolate mofetil, anti-CD25 monoclonal antibody (Basiliximab) and anti-thymocyte globulin (ATG Fresenius).

Results: all patients engrafted at median time of 21 days (range, 11-32) and 17 days (range, 11-26) to reach $\geq 0.5X10^9$/L neutrophil count and $\geq 20x10^9$/L platelet count. No primary or secondary graft failure occurred. At day 60 post-transplant all patients showed a full-donor chimerism. Grade I-II acute GvHD and mild/moderate chronic GvHD occurred in 6 and 2 patients, respectively. No patient died of transplant-related cause. Nine patients relapsed, at median of 90 (range, 28-691) days. Six early relapse occurred within 120 days of transplant. Marrow

relapse was observed in 6 patients and meningeal, cutaneous, soft tissue relapse was observed in other 3 patients, respectively. At 64 months after transplantation, the overall survival (OS) was 52% and the median of survival was not reached. The disease free survival (DFS) was 32% at 30 months after transplant and median DFS was 21 months. Probably due to the small sample size, the relapse was not influenced by disease status at transplant, association of NPM1 mutations, acute or chronic GVHD occurrence, rates of engraftment, previous chemotherapy courses, donor type, stem-cell source.
Conclusion: FLT3/ITD remains a poor prognostic factor even after HCT. The risk for early relapse after transplantation is high. However these results seem by far better than those obtained with chemotherapy, which has been followed by a median survival of only 2.5 months. Efforts are needed to explore early maintenance therapy with FLT3-ITD inhibitors in the post-transplant setting.
Disclosure of Interest: None declared.

P721
Outcome of karyotypic normal FLT3-ITD mutated AML patients treated intentionally with upfront alloHCT - single center experience

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Introduction: Patients with karyotypic normal FLT3-ITD mutated AML have dismal prognosis and the only curative option seems to be alloHCT. However, the timing of alloHCT is still matter of debate and some advocates HCT only beyond 1st CR. The consistent policy since 2003 in our centre is to refer all Flt3-ITD + AML pts to upfront alloHCT in CR1 (either related or unrelated).
Materials (or patients) and methods: With the aim to evaluate the outcomes of this strategy we have retrospectively analysed 50 consecutive adults with FLT3-ITD + AML allografted in our centre from 2003-2014 either with matched sibling (MSD, $n = 17$) or unrelated donors (UD, $n = 33$). Conditioning regimen was either RIC (Flu-MEL, $n = 36$) or myeloablative (BU-Cy, $n = 14$). The median age of cohort was 53 years (23-68). The original aim of our policy was to perform HCT in CR1 ($n = 39$), however in some patients due to various reasons (donor availability, disease resistance or patient preference), HCT was performed in more advanced stage ($n = 11$, 2xCR2, 2xPIF, 7xREL). Median interval from dg. to HCT was 5 months.
Results: With median follow up of 46 months (range 5-129) altogether 24 pts (48%) has died mainly of relapse ($n = 15$, 63%). The probabilities of OS and LFS at 3 years were 50% and 48%. Cumulative incidences of relapse (RI) and TRM at 3 years were 32% and 16%, respectively. All patients transplanted outside CR1 has died mainly of relapse (8/11), The median survival for transplanted in CR1 was not reached whereas for transplanted beyond CR1 was 4 months. The analysis has revealed that HCT at CR1 was the main factor significantly correlating with better OS (RR 0.3, $P = 0.253$), LFS (RR 0.359, $P = 0.001$) and lower RI (RR 0.251, $P = 0.0001$). The type of donor (related versus UD) as well as type of conditioning has no influence on OS and LFS, higher TRM associated with UD and BuCy was offset by lower relapse incidence. The presence of cGVHD was associated with improved OS and LFS (RR 0.2, $P = 0.0172$ and RR 0.3, $P = 0.0253$) due to the lower RI
Conclusion: Despite the limitations of a retrospective single center -based study, our study suggests alloHCT is a valuable therapeutic option for FLT3-ITD mutated AML patients and is able to provide durable remission. The benefit is most pronounced in patients allografted in CR1. Until prospective studies are completed, our study strongly supports to prioritize the allografting in CR1 regardless of donor type in this subset of AML patients.
Disclosure of Interest: None declared.

P722
The relapse in acute leukemia after the allogeneic hematopoetic stem cell transplantation: No approaches have been superior yet

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Introduction: The relapses after the transplantation have been about 20-50% of all the deaths. The best approaches in the postransplantation relapses have not been defined yet. These approaches include: The cessation of the present immunosuppresive treatment; donor lymphocyte infusion alone or with chemotherapy or with 5-azacytidine; second allo-HSCT from the same or an alternative donor; and radiotherapy alone or combination with systemic chemotherapy in the extramedullary relapses. In this retrospective study, we presented our center experiences in the approaches of relapses and/or progression in the patients with acute leukemia after allogeneic Hematopoietic Stem Cell Transplantation (ASCT).
Materials (or patients) and methods: We included total 133 patients (86 Male; 47 Female) with acute leukemia detected relapse and/or progression after ASCT between June 1990 and October 2013.Median age was 30 years (16-63 years) at the transplant time. Their diagnosis were 89 AML (12 secondary AML), 41 ALL and 3 biphenotipic leukemia. Median time of relapse and/or progression was 165 days (13-4713 days) after ASCT. Relapse types were as 11 extramedullary relapses (EMR) alone, 30 extramedullary with hematological relapses and 92 hematological relapses (HR) alone.
Results: Immunosupresive treatment with dose reduction or immediately was interrupted in 36 patients which relapsed in the first 100 days after the transplantation. In our cohort group, six patients died of the progressive disease without receiving any therapy. Most of the patient received chemotherapy alone ($n = 77$) or combination with DLI ($n = 39$). In chemotherapy group, 5-azacytidine (5-AZA) was given in 5 patients and 3 imatinib. Radiotherapy alone was applied in only two patients. We made second transplantation in 19 patients. After the year of 1999 the approaches including DLI or second allo-transplantation has increased. Chemotherapy alone ($n = 7$) or plus DLI ($n = 10$) had been used before the second transplantation. Only 4 patients prior to the second transplantation had a response to the treatment. The probabilities of one and two year-survival after the relapse were calculated as $25.1\% \pm 2.3\%$ and $11.3\% \pm 2.9\%$, respectively. The type of relapse (EMR alone, EMR plus HR versus HR alone) and leukemic types (AML vs ALL) did not effect on the survival after the relapse ($P = 0.95$ and 0.15, respectively).The survival after the relapse was better in 1st or 2nd remission compared with active or beyond 2nd remission at the first transplantation ($P = 0.02$). The early or late relapse ($<$ or $= 1$ year vs > 1 year) did not change the survival ($P = 0.8$). The patients underwent second transplantation for the relapse hadtended to be statistically better survival than the other approaches ($P = 0.08$).
Conclusion: Our treatment approaches in the relapses after the transplantation have been varying over the years. Although second transplantation compared to other treatment modalities seems to be a promising approach, any treatment modalities has not been superior in the leukemic relapse after the transplantation at all.
Disclosure of Interest: None declared.

P723

Stem cell transplantation from unrelated donors in adult ALL patients have comparable long term results than from matched related donors

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Introduction: In the GMALL studies very high risk patients (vHR) (Ph+ ALL) and high risk (HR) patients (pro B-ALL, B lineage ALL with high leukocyte counts at diagnosis or delayed CR, mature T ALL, prae T ALL, and patients with molecular failure or molecular relapse) are candidates for allogeneic SCT in CR1. Beyond CR1, all patients are candidates for allo SCT. Donors are HLA identical siblings, HLA compatible family donors, and HLA compatible unrelated donors. We report here our single centre data.

Materials (or patients) and methods: From 05/1995 to 12/2013, 211 adult ALL patients (132 male, 79 female) with a median age of 34 years (16-67) underwent allogeneic SCT. 133/211 patients (63%) were transplanted from compatible unrelated donors (MUD), 78/211 (37%) from matched related donors (MRD).

112 patients were transplanted in CR1 (Ph+ $n=42$; HR B lineage $N=42$; HR T lineage $n=28$). Donors were MUD in 69 patients (62%), MRD in 43 patients (38%). 37 patients were transplanted in CR2+CR3 (Ph+ $n=5$; B lineage $n=22$; T lineage $n=10$). Donors were MUD in 62%, MRD in 38%. 62 patients were transplanted with active disease, not in CR (Ph+ $n=13$; B lineage $n=36$; T lineage $n=13$) from an MUD (66%) or an MRD (34%).

Results: 88/211 patients (42%) are alive in CR between 12 months and 234 months after allogeneic SCT. 123/211 patients (58%) are dead. Causes of death were leukemia in 62/123 patients and TRM in 61/123 patients. Probability of survival for MUD at 5 years was 0.43, at 10 years 0.32, and at 13 years 0.32; for MRD 0.45, 0.40, and 0.32 (at 17 years). In CR1, probability of survival for MUD at 5 years was 0.55, at 10 years 0.41; for MRD 0.64 at 5 years, 0.60 at 10 years (not different). In CR2+3, no difference was found (MUD 0.35 at 5 years, 0.17 at 10 years; MRD 0.29 at 5 years, 0.21 at 10 years). In patients transplanted not in CR, there was also no difference between MUD and MRD (0.28 at 5 years, 0.23 at 10 years; and 0.19 at 5 years, 0.14 at 10 years).

Conclusion: Our long-term results show cure of high risk ALL patients. Since in advanced disease the relapse rate is high, patients should be transplanted earlier (e.g. with molecular failure). TRM decreased in the last years because of better supportive care and improved GvHD prophylaxis, but this cannot be shown in the long-term data.

Disclosure of Interest: None declared.

P724

Single Asian centre experience in haematopoietic stem cell transplantation for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia

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Introduction: Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) is found in 20% to 30% of adult ALL with a distinct clinical entity associated with dismal outcome when treated with conventional chemotherapy alone. There is a paradigm shift in the treatment outcome of Ph^+ ALL since the emergent of tyrosine kinase inhibitors (TKIs) although haematopoietic stem cell transplantation (HSCT) still remains an attractive curative option. In country with limited access to TKIs beyond first generation, we routinely treated our Ph^+ ALL patients with Imatinib in combination with chemotherapy as induction therapy prior to HSCT.

Materials (or patients) and methods: We evaluate the treatment outcome of Ph^+ ALL patients who had undergone HSCT after induction of chemotherapy in combination with Imatinib. All records of the patients were analyzed in a non-randomized retrospective study on 15th December 2014. Overall survival was estimated using the Kaplan-Meier method.

Results: A total of 68 patients diagnosed with Ph^+ ALL out of 229 patients with B lineage ALL (29.7%) from January 2007 to Dec 2014 in our centre. Only 36 of them (52.9%) managed to proceed with HSCT, with 32 (88.8%) allogeneic (of which 30 MRD and 2 MUD), 2 (5.6%) haploidentical and 2 (5.6%) autologous respectively. All the patients received full myeloablative CyTBI (12 Gy) regimen except haploidentical HSCT patients who were given our novel conditioning consist of FluBuTBI(2 Gy) with T cell repleted graft of cell dose around 3-5x10^6 CD34/kg among allogeniec patients as opposed to megadose of 10x10^6 CD34/kg in haploidentical HSCT setting, and conventional CSA/MTX as immunosuppressant for HLA-matched related donor with additional of ATG among matched-unrelated as well as haploidentical allograft. All the patients will receive Imatinib post transplant once the graft is stable. The median age of patients at transplant was 33.5 years (range 13 to 49). Male to female ratio was 1.25:1 with an ethnic composition of Malays 52.8%, Chinese 36.1%, Indian 8.4% and others 2.7%. All the patients underwent autologous and haploidentical HSCT were in complete remission (CR1) with MRD negative prior to transplantation. In contrast, for allogeneic cohort, only 28 of them were in CR1 (87.5%) and 2 in CR2 (6.3%) with total of 33.3% MRD negativity while 2 was in frank relapse prior HSCT. Amongst the 32 allogeneic HSCT cohort, the TRM was 18% (2 died from VOD, 3 died from severe sepsis prior engraftment and one succumbed from opportunistic infection in the first 100 days); 13 of them (40.6%) still alive in CR while the relapse mortality was 28.1% (involving 9 patients) and non relapse mortality (NRM) was 12.5% with 3 mortality as a result from GVHD. Grade II to IV acute GVHD developed in 35.7% of patients. The overall survival rate was 49% at 3 years for those underwent allogeneic HSCT. All the 4 patients underwent autologous and haploidentical HSCT are still in complete remission with the longest follow up of 5 years.

Conclusion: The analysis of a small cohort of our patients with Ph^+ ALL suggests a trend towards a favorable outcome with HSCT. Autologous and haploidentical HSCT represent a feasible alternative for allgeneic stem cell transplantation in Ph^+ ALL patients who achieved CR with MRD negativity.

References: 1. Biol Blood Marrow Transplant. 2000;6(6): 597-603

2. Haematologica.2014 Jan;99(1):111-5

Disclosure of Interest: None declared.

P725

Factors associated with improving of 5-years overall survival in patients with acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation

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Introduction: The aim of our study was estimate the main factors predicting treatment results in allogeneic hematopoietic stem cell transplantation (alloHSCT) in adolescents and adults with acute myeloid leukemia (AML).

Materials (or patients) and methods: We analyzed the results of 255 AML patients (pts) after alloHSCT – matched family donor (MFD) – 78(31%), matched unrelated donor (MUD) – 175(69%). The median age was 33 (10-69) years. 202 (80%) cases of AML were *de novo*, 51 (20%) pts had secondary AML. At the moment of alloHSCT 94 pts (37%) were in complete remission (CR) 1, 62 (25%) – in CR2 and 97 (38%) pts had active disease (AD). At diagnosis cytogenetic analysis was performed in 191 (76%) cases, 109 (57%) of intermediate and 54 (28%) of unfavorable risk. Myeloablative conditioning (MAC) was administered in 73 (29%) pts, reduced intensity conditioning (RIC), in 180 (71%) pts. 82 (32%) pts were transplanted with bone marrow (BM), 168 (66%) – with peripheral blood stem cells (PBSC).
Results: Five-years OS after alloHSCT in CR1 was 63%, in CR2–36%, AD – 13% ($P<0.001$) and doesn't depended from cytogenetic risk: CR1, high risk – 41%, CR1, standard risk – 64% ($P=$ NS). The relapse incidence (RI) after alloHSCT in CR1, high cytogenetic risk was 24% and CR1, standard risk – 20%, CR2, high risk - 31% and CR2, standard risk – 31%. According to conditioning regimens 5-years OS was 79% in CR1 pts after MAC and 60% after RIC alloHSCT, in CR2 pts – 49% and 28% ($P=$ NS). There was no significant dependence of 5-years OS, neither on donor type (related/unrelated) - 52% and 54%, nor on transplant source (BM/PBSC) 52% and 49% ($P=$ NS). However, RI in CR1 pts after MFD was 35%, after MUD – 12% ($P=0.02$), in CR2–44% and 20% ($P=0.05$). Incidence of acute graft-versus-host disease (aGVHD) grade 3-4 after alloHSCT from MFD was 20%, after alloHSCT from MUD – 35% ($P=$ NS), the cumulative incidence of extensive chronic GVHD (cGVHD) was 9,8% and 38,4% ($P=0.001$). The main cause of death after MFD alloHSCT was disease progression (67%), while in MUD alloHSCT it was aGVHD (25%) and infections (25%). The risk of relapse was lower in pts with cGVHD ($P=0.01$). Multivariate analyses has demonstrated that the risk of relapse was higher in patients who achieved full donor chimerism later than by day + 30 ($P=0,01$) and did not have aGVHD ($P=0.02$), whilst lower risk of relapse was in cases of aGVHD grade 1 or 2 ($P=0.03$ and $P=0.02$ respectively), MUD, and PBSC as source of transplant ($P=0.02$).
Conclusion: Our data confirm that CR1 is the optimal time for HSCT in AML patients. Conditioning regimen and the source of the graft did not affect on the OS with the exception of the increased risk of relapse after MFD alloHSCT. The risk of relapse is lower in full donor chimerism up to day + 30, aGVHD and cGVHD that is higher in PBSC and MUD comparing to MFD alloHSCT.
Disclosure of Interest: None declared.

P726
Risk factor analysis for the outcome of high-risk AML patients treated with the FLAMSA conditioning regimen: strong impact of extramedullary involvement in patients with active disease

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Introduction: Introduction: Patients with secondary AML, high risk cytogenetics as well as remaining disease burden provide an increased risk of leukemia relapse. This may be addressed at the time of transplant by a sequential conditioning regime including primary a chemotherapy followed by reduced intensity conditioning (RIC) for HSCT. To determine clinical risk factors for outcome in high risk AML patients after conditioning with FLAMSA a retrospective single center analysis was performed.
Materials (or patients) and methods: Materials (or patients) and Methods: In this analysis, 84 patients diagnosed with de novo AML ($n=67$) or secondary AML ($n=17$) treated between 2000 and 2012 were included. For the purpose of this study high-risk disease was defined as patients with high-risk cytogenetics in first complete remission (CR), secondary AML in first CR, patients in second CR, primary induction failure and chemo-refractory relapse. The conditioning-regimen consisted of fludarabine (4 x 30 mg/m^2), cytarabine (4 x 2 g/m^2), and amsacrine (4 x 100 mg/m^2), followed 4 days later by total body irradiation (TBI) with 4 Gy, cyclophosphamide, and antithymocyte globulin. Outcome was analyzed with respect to overall survival (OS), cumulative incidence of relapse (CIR) and death (CID).
Results: Results: The median age at HSCT was 48.7 years, $n=46$ (55%) of the patients were male. Before HSCT 13 (16%) patients achieved a first complete remission (CR) during first line induction therapy, 12 (14%) patients a second CR after relapse and 59 (70%) patients were refractory (primary refractory $n=31$ and refractory relapse $n=28$). In 24 patients (29%) extramedullary disease was present at HSCT (7 (25%) chloroma, 16 (67%) meningeosis and 2 (8%) with both). None of the patients presented exclusively with extra-medullary disease at HSCT.
The median OS after HSCT was 12.1 months (CI 95% range, 6.7-17.6 months) with survival rates at 1, 2 and 4 years of 51%, 35% and 24% respectively. There was no significant difference in OS, CIR and CID according to sex, age, donor type, FLT3-ITD status and cytogenetics. The hematologic remission rate of refractory patients after HSCT was 84% (4 patients died during HSCT). CID and CIR for patients in CR and no-CR after 12 months was 24%, 59% and 16%, 50% respectively. Patients transplanted in CR had a median two year OS rate of 60% in comparison with 24% for refractory patients. The strongest factors for dismal prognosis in a multivariable analysis were the absence of a CR at time of HSCT and a concurrent extramedullary disease ($P=0.017$). The median OS of these patients was 3.6 months in comparison to patients with exclusive active bone marrow disease with a median OS of 13 months ($P<0.0001$, HR 3.75).
Conclusion: Discussion: FLAMSA-RIC followed by allogeneic HSCT enables acceptable overall survival rates in high risk AML patients. However, patients with active leukemic disease especially with concurrent extra-medullary disease have a dismal prognosis which highlights the need for early detection combined with prior reduction of the leukemic burden to improve survival.
Disclosure of Interest: None declared.

P727
Allogeneic Transplantation in Elderly Patients with AML and MDS Comparing Reduced-Intensity Conditioning with FLAMSA-Busulfan versus Fludarabine/BCNU/Melphalan

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Introduction: Different reduced-intensity conditioning (RIC) regimens are available for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in elderly patients.
Materials (or patients) and methods: Here, we retrospectively evaluate the outcome of sequential therapy employing RIC with fludarabine 30 mg/m^2, cytarabine 2 g/m^2 and amsacrine 100 mg/m^2 for 4 days (FLAMSA; Schmid C et al. JCO 2005) followed by busulfan 10x0.8 mg/kg (FLAMSA-Bu) compared to RIC utilizing fludarabine 5x30 mg/m^2, carmustine (BCNU) 2x150 mg/m^2 and melphalan 110 mg/m^2 (FBM; Marks R et al. Blood 2008) in elderly patients treated consecutively at our institution between July 2005 and October 2012.
Results: We analyzed the course of 114 patients (pts) with acute myeloid leukemia (AML; $n=99$) or myelodysplasia (MDS;

$n = 15$) aged ≥ 59 years with 59 pts aged ≥ 66 years who were treated with either FLAMSA-Bu ($n = 66$; $n = 24 \geq 66$ years) or FBM ($n = 48$; $n = 35$ ≥ 66 years). All patients received serotherapy with anti-thymocyteglobuline (ATG). Median patient age was 66 years for the entire cohort (68 years FBM; 64 years FLAMSA-Bu). 36 patients (75%) of the FBM and 42 patients (63%) of the FLAMSA-Bu group suffered from high risk disease defined as relapsed or refractory AML or refractory anemia with excess of blasts in transformation (RAEB-T). The hematopoietic cell transplantation comorbidity index (HCT-CI) was higher for the patients of the FBM group than for the FLAMSA-Bu group with 26 (54%) versus (vs) 24 patients (36%) scoring ≥ 2 (p 0.085). Graft source after conditioning with FBM/FLAMSA-Bu was bone marrow (1/2), G-CSF mobilized peripheral blood stem cells (40/62) and double-umbilical cord-blood (7/1). In 23 pts (20%) HLA-matched related and in 91 pts (80%) HLA-matched unrelated donor transplantation was performed. Incidence of severe acute (III-IV) and chronic GvHD was 22.9%/16.6% for FBM vs 18.2%/19.7% for FLAMSA-Bu, respectively. After conditioning with FBM 2/48 pts vs 9/66 pts after FLAMSA-Bu were diagnosed with a secondary malignancy (p 0.08). Non-relapse mortality after 12 months was 26.8% for FBM versus 25.2% for the FLAMSA-Bu group. Incidence of relapse after FBM vs FLAMSA-Bu conditioning was 22.9% vs. 15.2% after 1 year and 31.3% vs 16.7% after 2 years. After a median follow up of 31.4 months (range 4.4-97.5) estimated overall survival (OS) and relapse-free survival (RFS) after 2 years was 55.4% and 51.4% for the FBM vs 58% and 56.7% for the FLAMSA-Bu group, respectively. Analyzing different subgroups, FBM conditioning might be favorable for pts aged ≥ 66 years when suffering from high risk AML ($n = 26$): Within this group 1-year OS after FBM vs FLAMSA-Bu was 71.4% vs 66.7% (p 0.58) and 1-year RFS was 71.4% vs 58.3% (p 0.59), respectively. Notably, for pts at highest risk (aged ≥ 66 years and suffering from secondary or therapy-related AML; $n = 24$) the benefit of FBM conditioning becomes more remarkable: 1-year OS after FBM vs. FLAMSA-Bu was 62.5% vs 37.5% (p 0.26) and 1-year RFS 54.2% vs. 37.5% (p 0.17).

Conclusion: Both conditioning regimens are feasible and provide similar rates of acute toxicity, NRM and GvHD. There might be evidence for a benefit of conditioning with FBM for the subgroup of "the oldest patients at highest risk".

Disclosure of Interest: None declared.

P728

Outcome of allogeneic hematopoietic stem cell transplantation in patients with KMT2A (MLL)-related leukemia, depending on number of transplanted CD34 + cells

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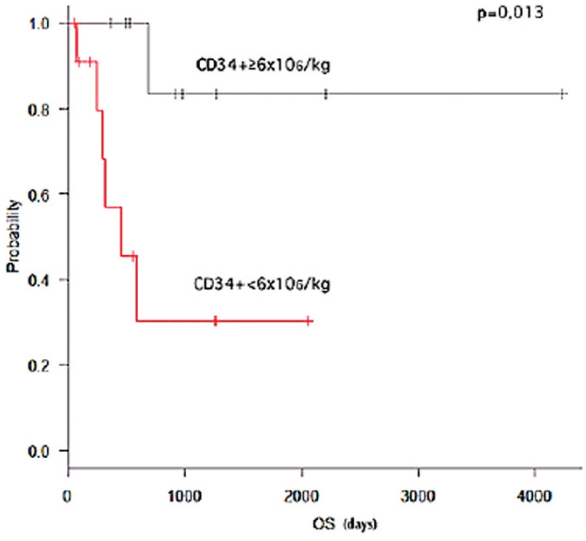
Introduction: *KMT2A (MLL)* gene rearrangements are found in all age groups of acute leukemia (AL) patients (pts), more often in infant. Independently of their associations with other high-risk features at presentation *KMT2A* rearrangements are in most cases predictive of poor clinical outcome [Tamai and Inokuchi, 2010]. Although HSCT is the only curative treatment for *KMT2A* + AL, most of these patients relapsed. To elucidate the problem we have analyzed outcomes of alloHSCT for a mixed cohort *KMT2A* + AL pts.

Materials (or patients) and methods: In this study, outcomes of alloHSCT, which were performed in a single institution between 2003 and 2014 for patients with *KMT2A* + AML and ALL were analyzed. The pts were selected according to the following criteria: (i) diagnosed as AL with *KMT2A* rearrangements; (ii) alloHSCT was performed in CR1 or CR2. All patients and transplantation characteristics are listed in Table I.

Results:

Leukemia, n (%)	
AML	15 (68. 2)
ALL	7 (31.8)
Patient sex, n (%)	
Male	8 (36.4)
Female	14 (63.6)
Age at HSCT, median, (range) years	14.5 (1.3-37)
Cytogenetics,n(%)	
t(4;11) KMT2A-AFF1	7(31.8)
t(9;11) KMT2A-MLLT3	8 (36.4)
t(11;19) KMT2A-MLLT1	2 (9.1)
Other *KMT2A*	4 (18.2)
Time from diagnosis to HSCT, median (range) days	452 (108–1348)
Clinical stage at HSCT, n (%)	
CR1	16 (72.7)
CR2	6 (27.3)
HSC source, n (%)	
Bone marrow	16 (72.7)
Peripheral blood	6 (27.3)
Conditioning regimen, n (%)	
MA	13 (59.1)
Non-MA	9 (40.9)
Donor type, n (%)	
HLA-id sibling	7 (31.8)
Matched unrelated	10 (45.5)
One-Ag mismatched unrelated	2 (9.1)
Haploidentical	3 (13.6)
Number of transplanted CD34 + cells median (range), x10^6/kg	6.1 (1.6-14.9)



The probabilities of overall survival (OS) and event free survival were 57.0% (CI, 28.9–77.5%) and 60.8% (CI, 34.0–79.5%) at 10 years, respectively. Pts receiving more than the median CD34 + cells had higher OS compared to the recipients receiving less than the median CD34 + cells (83.3% vs. 30.3%, $P = 0.013$) as a result of lower NRM (48.8% vs 0.0% $P = 0.01$) in the former group (Figure1). Other factors including pts sex, age, disease type, partner genes of *KMT2A* rearrangements, stem cell source, HLA disparity and conditioning regimen were not associated with survival. In multivariate analysis, more CD34 + cells infused were found to be the only predictor for higher OS with HR of 11.42 (95% CI, 1.33–98.4, $P = 0.027$).

Conclusion: A number of transplanted CD34 + cells is a one of the important transplant characteristics that has a significant prognostic value for transplant patients AL with *KMT2A* gene rearrangements in CR1 and CR2.

Disclosure of Interest: None declared.

P729

Sorafenib as a safe bridge to immunotherapy in patients with FLT3-ITD relapsed AML after allogeneic HSCT: a single center experience in 14 patients

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Introduction: Relapsed acute myeloid leukemia after allogeneic HSCT have a dismal prognosis. Unfortunately, a few therapeutic options are available for this patients. Sorafenib is a tyrosine kinase inhibitor with activity against VEGF, RAF, and FLT3-ITD which has been approved by the FDA for treatment of some solid tumors and has been used off-label and in several exploratory trials in AML with variable results

Materials (or patients) and methods: Here we report a single center experience in 14 FLT3-ITD AML patients (pts) relapsed after allogeneic HSCT that have been treated with sorafenib as salvage therapy between June 2012 and December 2014. After signing off-label informed consent, 13 pts started sorafenib at the 400 mg bid dose and 1 pt received a half dose (200 mg bid). In 3 patients concomitant treatments were associated to sorafenib: azacytidine (1 pt), azacytidine + donor lymphocyte infusion (DLI) (1 pt) or allogeneic PBSC boost (1 pt).

Results: The median follow up time was 89 days (range: 11 to 469 days). Nine pts had blasts in the PB before starting sorafenib; all of them cleared blasts from PB in a median time of 9 days (range: 2 to 27 days). The hematologic ORR was 50% (2 CR + 1 CRi + 4 PR) and 8 patients are alive. Among 7 patients in PD, 3 received an allogeneic PBSC boost without efficacy and 2 were candidated to a second allogeneic transplant achieving a CR. In 4 cases a dose adjustment was necessary by grade IV hematological toxicity (neutropenia and thrombocytopenia) and grade III skin toxicity (*hand-foot syndrome*). The median drug exposure time was 53 days (range: 12 to 384 days). Of note, *hand-foot syndrome* associated in all 3 patients to a complete remission of leukemia.

Conclusion: Sorafenib is an efficient therapy in FLT3-ITD AML relapsed after allogeneic transplantation and can be considered a potential tool to bridge, at an acceptable toxicity, to a subsequent immunotherapy such as DLI or second transplantation.

Disclosure of Interest: None declared.

P730

Impact of Pre-transplant Serum Ferritin on Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation for Acute Leukemia

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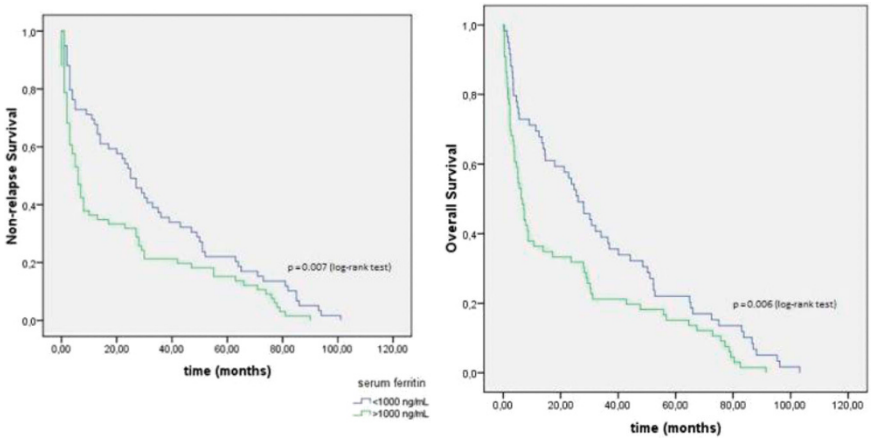
Introduction: Iron overload has been linked to several adverse outcomes including lower overall and relapse-free survival (OS and RFS) among allogeneic hematopoietic stem cell transplantation (HSCT) patients. Effects on the incidence of relapse and acute and chronic graft-versus-host disease (aGVHD, cGVHD) have been reported. In this study, we aimed to investigate these associations by using pre-transplant serum ferritin (PTSF) as a surrogate marker for iron overload.

Materials (or patients) and methods: Among 257 patients who underwent allogeneic HSCT in Ankara University Bone Marrow Transplantation Unit for acute leukemia between January 2005 and December 2012, serum ferritin levels within 3 months before the procedure was available for 125 patients (48.6%). Medical records of these patients were retrospectively reviewed for demographic, disease and transplant characteristics. Patients with high serum ferritin (>1000 ng/mL) were compared to patients with lower serum ferritin (<1000 ng/mL) with respect to HSCT end-points including OS, RFS, incidence of relapse, aGVHD and cGVHD, engraftment, post-HSCT invasive fungal infection (IFI) and transplantation related mortality (TRM). Chi-square and Mann-Whitney U test were used for analyses of variance. Survival estimates were calculated with Kaplan-Meier method. $P<0.05$ was considered statistically significant.

Results: The median age of the patients at time of HSCT was 35 (16-67) and 56.8% ($n=71$) were male. Acute leukemia subgroups involved acute lymphoblastic leukemia ($n=29$), acute myeloid leukemia (AML) ($n=85$), myelodysplastic syndrome (MDS) related AML ($n=9$), and biphenotypic acute leukemia ($n=2$). The source of HSCT was peripheral blood in 88.8% ($n=111$). Other sources were cord blood ($n=8$) and bone marrow ($n=5$). Conditioning regimen was myeloablative in 86.4% ($n=108$) and reduced intensity in 13.6% ($n=17$). GVHD prophylaxis was given with CsA + Mtx in 85.6% ($n=107$) and with CsA + MMF in 11.2% ($n=14$). PTSF was >1000 ng/mL in 52.8% ($n=66$). Concurrent serum CRP was >10.0 ng/mL in 37.6% ($n=44$). The rate of disease progression, aplasia and early TRM in high PTSF group was 16.7% and

[P730]



significantly higher compared to lower PTSF group (5.1%) ($P = 0.04$, $c^2 = 4.202$). Post-HSCT IFI rate was significantly higher in high PTSF group (50.8% vs 25.4% $P = 0.004$, $c^2 = 8.374$). Overall mortality and TRM were also higher in the high PTSF group [60.6% vs 42.4% ($P = 0.04$, $c^2 = 4.149$) and 33.3% vs 13.6% ($P = 0.04$, $c^2 = 4.149$) respectively]. Relapses were similar between two groups ($P = 0.7$, $c^2 = 0.132$). PFS and OS were worse in high PTSF group ($P = 0.007$ and $P = 0.006$, log-rank test; respectively). AGVHD and CGVHD rates were similar between groups ($P = 0.8$, $c^2 = 0.101$ and $P = 0.1$, $c^2 = 2.565$; respectively). Engraftment failure was more frequent in high PTSF group, however, the difference was not statistically significant [12.1% vs 3.4% ($P = 0.07$, $c^2 = 3.227$)].

Conclusion: Consistent with previous observations higher PTSF levels are associated with poor PFS and OS in this population. Post-HSCT IFI risk also increases with increasing PTSF. Further research is warranted to investigate the benefit of interventions aiming at lower PTSF levels.

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Disclosure of Interest: None declared.

P731

Prognosis of Acute Leukemia Patients with Active Disease Undergoing Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT)

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Introduction: Prognosis of acute leukemia patients undergoing allo-HSCT without complete remission is poor. Yet it offers the only curative option for these group of patients. We present here our experience with this group of patients.

Materials (or patients) and methods: Among 257 patients who underwent allogeneic HSCT in Ankara University Bone Marrow Transplantation Unit for acute leukemia between January 2005 and December 2012, active disease was present in 43 patients (16.7%). Medical records of these 43 patients were retrospectively reviewed for demographic, disease and transplant characteristics. Patients were subgrouped acccording to their bone marrow blast percentage before the HSCT as having a blast percent of $< 30\%$ and $> 30\%$. HSCT end-points including overall survival (OS), leukemia-free survival (LFS), incidence of relapse, aGVHD and cGVHD, transplantation related mortality (TRM) according to pre-HSCT blast percents, type of conditioning regimen, the presence of pre-HSCT cytoreductive chemotherapy.

Results: The median age of the patients at time of HSCT was 30 (16-61) and 72.1% ($n = 31$) were male. Acute leukemia subgroups involved acute lymphoblastic leukemia ($n = 13$), acute myeloid leukemia (AML) ($n = 24$), myelodysplastic syndrome (MDS) related AML ($n = 3$), and biphenotypic acute leukemia ($n = 3$). Conditioning regimen was myeloablative in 62.8% ($n = 27$) and reduced intensity in 37.2% ($n = 16$). GvHD prophylaxis was given with CsA + Mtx in 60.5% ($n = 26$) and with CsA + MMF in 27.7% ($n = 13$). The rate of disease progression, aplasia and early TRM were lower among patients with pre-transplant bone marrow blasts $< 30\%$, however, the difference was not statistically significant (7.6% vs 30.0% $P = 0.1$, $c^2 = 2.529$). Overall mortality was higher in the higher blast group (93.3% vs 61.5% $P = 0.009$, $c^2 = 6.727$), however overall TRM and relapses were similar between two groups. LFS after allogeneic HSCT was similar ($P = 0.1$, log rank test), whereas OS was significantly shortened in higher blast group ($P = 0.02$, log rank test). Myeloablative conditioning regimens was significantly superior to reduced intensity regimens in view of OS and LFS ($P = 0.04$ and $P = 0.04$, log rank test; respectively). Among patients conditioned with a reduced intensity regimen 81.3% received a cytoreductive regimen (FLAG-IDA, FLAMSA) prior to conditioning, whereas, only 18.5% of patients conditioned with a myeloablative regimen received prior cytoreductive therapy. Cytoreductive treatment prior to the condidioning regimen had an impact on the LFS ($P = 0.03$), but not OS ($P = 0.4$).

Conclusion: Higher pre-HSCT blast percentage is related to a poorer prognosis after allogeneic HSCT for acute leukemias. In this patient population the results of HSCT using reduced intensity conditioning regimens are significantly worse and addition of pre-HSCT cytoreductive therapy cannot overcome their unfavorable effects. Hence, myeloablative regimens should be preferred, whenever possible.

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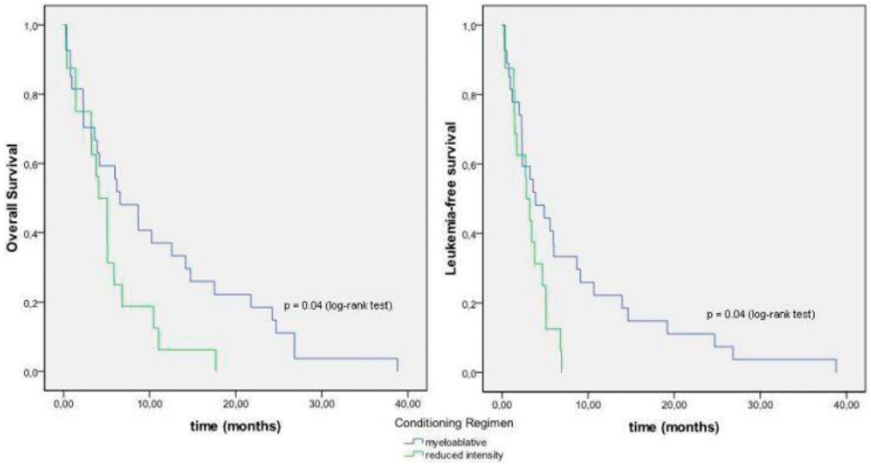
Disclosure of Interest: None declared.

[P731]



P732

Efficiency of hypomethylating agents after allogenic hematopoietic stem cell transplantation in acute myeloid leukemia and myelodisplastic syndrome

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Introduction: The aim of this study was to estimate overall survival (OS) and relapse incidence (RI) of patients with AML and MDS after allogenic hematopoietic stem cell transplantation (alloHSCT), receiving 5-azacitidine (5-aza).
Materials (or patients) and methods: We evaluated outcomes of 45 patients with myeloid malignancies (AML 84% (38), MDS (RAIB I-II 6, RCMD 1) 16% (7)) treated with 5-aza after alloHSCT. 8 patients were grafted from matched family donor, 30 - unrelated donor (matched – 25, mismatched – 5), 7- haploidentical. Median age was 56 years, range 1-67, 28 male, 17 female. Conditioning regimen was myeloablative in 27% (12) cases, reduced intensity conditioning was used in 73% (33) cases. Post-transplantation cyclophosphamide was used as a prophylaxis of "graft-versus-host-disease" in 42% cases (19), antithymocyte globulin – in 37% (17) cases. For 55% (25) patients transplantation was performed as a salvage therapy. In 62% (28) cases the administration of 5-aza was prophylactic, because of the high relapse risk: active disease at the moment of alloHSCT or the unfavorable cytogenetics. Preemptive 5-aza was administered to 19% (8) patients with minimal residual disease or mixed chimerism after alloHSCT. Median time for administration of 5-aza in preemptive and prophylactic groups varied day + 30 – day + 60 after alloHSCT; the main study inclusion criterion was the recovery of hematopoiesis; 5-aza was administered 35 mg/m2/daily, 5 days of 28- day cycle, 4 cycles. 5-aza was combined with donor lymphocyte infusion in 23% (10) cases. In case of relapse (bone marrow blasts > 5%) 5-aza was used for 19% (8) patients, median time of administration was day + 84 (33-149).
Results: One year overall survival (OS) in the prophylactic and preemptive groups was 74% (95% CI 47-99%) and 56% (95% CI 8-99%), respectively ($P < 0.001$). OS for patients who were not in remission at the start of the therapy is 0% (95% CI 28-52) (median of observation 3 months). Relapse incidence (RI) at the first year was 47% (95% CI 24-66%) and 45% (95% CI 7-75%) for prophylactic and preemptive administration ($P =$ NR), respectively. 5-aza therapy was well-tolerated, in all groups no cases of non-relapse mortality were observed.
Conclusion: 5-aza therapy in the post-transplant period for patients with high risk of relapse has encouraging results. Optimization of treatment scheme may be an option to improve results. 5-aza has low toxicity and can be used in early post-transplant period.
Disclosure of Interest: None declared.

P733

Validation of the EBMT Risk Score and HCT-CI in Adult Patients with Acute Myeloid Leukemia Undergoing Allogeneic Hematopoietic Stem Cell Transplantation in Taiwan

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Introduction: The European Group for Blood and Marrow Transplantation (EBMT) risk score and Hematopoietic cell transplantation comorbidity index (HCT-CI) were two commonly used scoring systems to predict the outcome and TRM for patients undergoing allogeneic hematopoietic stem cell transplantation (Allo-HSCT). The goal of this study is to investigate whether the prognostic values of the EBMT risk score are reliable and valid for adult patients with acute myeloid leukemia who undergo Allo-HSCT in a single center in Taiwan.
Materials (or patients) and methods: The medical records of the 400 adult patients with acute myeloid leukemia who underwent Allo-HSCT at National Taiwan University Hospital between the year of 1992 and 2013 were retrospectively collected following the EBMT Registry data collection forms and manuals. EBMT risk score and HCT-CI were calculated as described. Overall survival probability (OS) and non-relapse mortality rate (TRM) were estimated by the Kaplan-Meier method. Univariate and multivariate analysis were performed using Cox proportional hazard regression model. All of the parameters were evaluated the Cox proportional hazard assumption. This study was approved by the hospital Research Ethics Committee.
Results: The 6- yr OS rate in score 0-1 was 70.3%, followed by 39.5% in score 2-4 and 26% in score 5-7, showing a significant difference ($P < 0.001$). The TRM rates in score 0-1, score 2-4 and score 5-7 were 14.5%, 34.5% and 39.4% ($P = 0.002$), respectively. The EBMT risk score was identified as an independent prognostic factor for OS and TRM according to univariate and multivariate analysis (Table 1). The other independent risk factors include: unfavorable cytogenetics, donor age, and white blood count at diagnosis. In the initial assignment of HCT-CI, pulmonary and hepatic risk score were unexpectedly

[P733]

Table 1. Multivariate analysis of OS and TRM

	Patients No. (%)	OS		TRM	
		HR (95%CI)	p	HR (95%CI)	p
EBMT score					
0-1	68 (17)	1		1	
2-4	228 (57)	3.057 (1.73-5.40)	<0.001	2.634 (1.30-5.36)	0.007
5-7	104 (26)	4.808 (2.63-8.79)	<0.001	3.568 (1.65-7.71)	0.001
Cytogenetics before HSCT					
Favorable/Intermediate	346 (87)	1		1	
Unfavorable	54 (13)	2.414 (1.65-3.52)	<0.001	2.291 (1.40-3.75)	<0.001
Donor age	400 (100)	1.021 (1.01-1.03)	0.001	2.801 (1.32-5.95)	0.007
WBC at diagnosis	400 (100)	1.115 (1.02-1.23)	0.023		
HCT-CI					
Low risk	140 (35)	1		1	
Intermediate risk	135 (34)	0.927 (0.65-1.32)	0.677	0.739 (0.45-1.22)	0.236
High risk	125 (31)	1.034 (0.72-1.48)	0.854	0.984 (0.61-1.59)	0.948

high, after adjusted the carbon monoxide diffusing capacity for hemoglobin concentration, and excluded those liver function impairment due to chemotherapy or drugs, the HCT-CI was still not correlated with OS and TRM, while the EBMT risk score was better in predicting OS, TRM.
Conclusion: Our study showed that the EBMT risk score can reliably predict transplant outcomes for Chinese patients undergoing Allo-HSCT. Other factors, including cytogenetics before transplantation, donor age, and white blood count at diagnosis were significantly related with OS and TRM in our study. Further study to develop a more precise predictive scoring system is now in progress.
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Disclosure of Interest: None declared.

P734
Impact of FLT3-ITD mutations on the outcome of patients allografted with partial T-cell depleted grafts for AML in first complete remission with normal karyotype

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Introduction: FLT3-ITD mutations are associated with poor prognosis in acute myeloid leukemia (AML). Chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice because it increases overall survival (OS), progression-free survival (PFS) and decreases relapse incidence (RI) when compared with chemotherapy alone. Partial T-cell depletion (TDEP) reduces graft-versus-host disease (GvHD) incidence and may increase quality of life but may be associated with increased risk of relapse. Impact of FLT3-ITD mutations on outcomes has not yet been evaluated for partial TDEP in AML post-allogeneic HSCT.
Materials (or patients) and methods: We analysed 37 consecutive patients allografted between 2003 and 2013, for AML with normal karyotype in first complete remission (CR1). 51% were female and median age was 55 years (range: 22-67). All patients were allografted with peripheral blood stem cells from identical siblings (35%), matched unrelated donor (46%) and mismatched unrelated donor (19%). 54% of patients received a myeloablative and 46% a reduced intensity conditioning. Patients received grafts TDEP in vitro with CAMPATH-1H followed by an add-back of $100x10^6$/kg donor T cells on day +1. GvHD prophylaxis consisted of calcineurin inhibitor ± methotrexate, or mycophenolate mofetil. We have compared 5-year OS, PFS and RI for patients with FLT3-ITD ($n=8$) and FLT3wt ($n=29$).
Results: 5-year OS for patients with FLT3-ITD was 54 ± 40% and with FLT3wt was 59 ± 19% ($P=0.82$). 5-year PFS for FLT3-ITD was 57 ± 38% and FLT3wt was 58 ± 18% ($P=0.89$) and 5-year RI was 43 ± 38% and 30 ± 20% for FLT3-ITD and FLT3wt, respectively ($P=0.43$) (see figure). Acute GvHD grade 2-4 was experienced by 11% of patients and chronic GvHD by 16%.
Conclusion: We did not observe differences in OS between FLT3-ITD and FLT3wt patients. Moreover, there is no increase of RI or decrease of PFS in patients with FLT3-ITD. These results suggest the feasibility of partial TDEP in CR1 AML with normal cytogenetic, without hampering outcomes. Moreover, use of partial TDEP reduces acute and chronic GvHD incidence, which may increase the quality of life of patients. However our results should be interpreted with caution due to the low number of patients and the retrospective nature of the study and need to be confirmed in a largest prospective cohort.
Disclosure of Interest: None declared.

P735
Abstract Withdrawn

P736
Chemotherapy followed by donor stem cell infusion as induction treatment for relapsed acute leukemia after allogeneic stem cell transplantation: Cryopreserved stem cells or the second donation?

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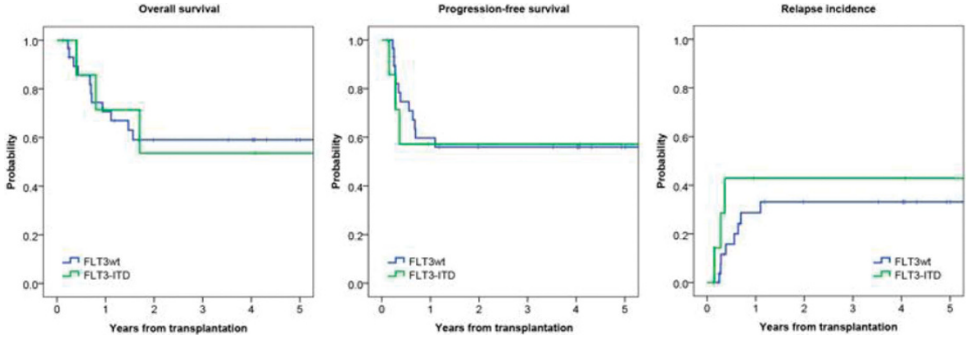
Introduction: Leukemia relapse becomes one of the leading causes of death after allogeneic hematpoietic stem cell transplantation (allo-HSCT). It is important to achieve complete remission again for it lays the foundation for further treatment such as second transplantation, immunotherapy etc. Recently chemotherapy followed by donor stem cell infusion (DSI) instead of unstimulated donor lymphocyte infusion (DLI) to treat relapsed leukemia after allo-HSCT has been associated with high remission rate. Donor stem cells have 2 sources: cryopreserved DSI and the second donation from the same donor. Which source of DSI will benefit the relapsed recipient more remains unknown.
Materials (or patients) and methods: 34 patients who relapsed acute leukemia after allo-HSCT were admitted. 21

[P734]



[P736]

Characteristics	CR P
Univariate analysis	
Age	0.789
Gender	0.876
Diagnosis	*0.049*
Disease status pre-HSCT	0.634
HLA-mismatch	0.213
BM blast at the time of relapse	0.765
Interval from HSCT to relapse	*0.025*
Acute GVHD of 2-4 grade post chemotherapy plus DSI	0.543
Chimerism at the time of relapse	0.651
CD34+ cells in DSI	0.433
CD3+ cells in DSI	0.475
MNCs in DSI	0.561
Chemotherapy plus DSI (Cryopreserveds vs second donation)	0.807
Univariate analysis	
Diagnosis	*0.025*
Interval from HSCT to relapse	*0.047*

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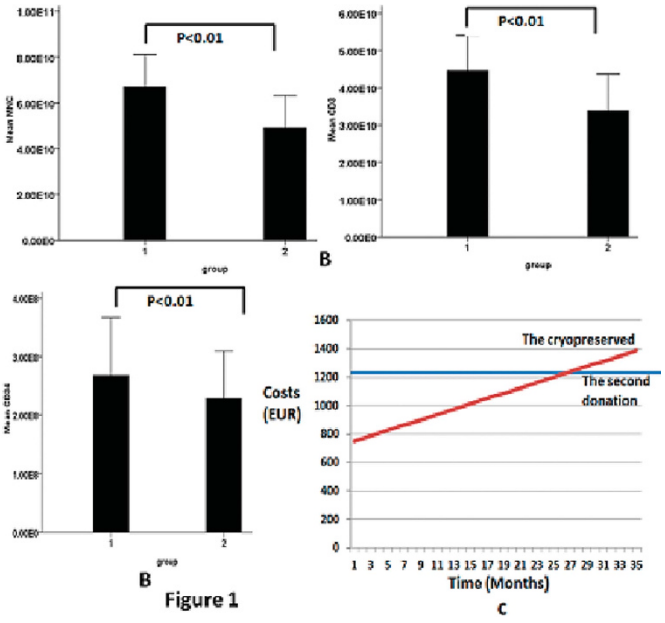

Figure 1

patients received chemotherapy followed by cryopreserved DSI, remaining 13 received chemotherapy followed by DSI from the second donation. We compared CR rate after induction treatment and hematopoietic reconstitution between 2 groups. Factors associated with CR rate were analyzed. We also explored the costs between the 2 sources of DSI. Finally we compared the safety and yield harvests between the first and second stem cell donation.

Results: CR rates were 42.9% and 53.8% in patients receiving chemotherapy followed by cryopreserved DSI and patients received chemotherapy followed by DSI from the second donation respectively ($P = 0.41$). Neutrophil recovery time were 12.5 + -2.2 days vs 13.6 + -3.4 days ($P = 0.09$) respectively. Platelet recovery time were 13.9 + -4.9 days vs 14.7 + -4.6 days ($P = 0.743$) respectively. Both univariate and multivariate analysis demonstrated diagnosis and interval from HSCT to relapse are 2 factors associated with CR (Fig1a). The mean total CD34 + cells, PBMCs and CD3 + T cells were significantly different between the first and second donation($P < 0.01$, see Fig1b) while the doses per kg recipient were all enough from both the first and second donations. All the donors both in the first donation and second donation have no serious adverse reaction. The cost of the second stem cell donation is about 1250EUR. The cryopreserved fee for the first time is 750EUR then 0.625EUR per day. The cost comparison of the cryopreseverved and the second donation are shown in Fig1C. From the Figure we can see that patients relapsed after 27months will have less cost if they use the second donation otherwise cryopreserved stem cells will have less cost.

Conclusion: There was no difference of CR rate between cryopreserved DSI and DSI from the second donation which meant both DSI can benefit the relapsed patients after allo-HSCT. The second donation from the same donor is feasible. For patients who relapse with estimated time of less than 27 moths cryopreserved DSI might be administered and for patients who relapse with estimated time of longer than 27months DSI from the second donation might be administered.

Disclosure of Interest: None declared.

Autoimmune diseases

P737

Occurrence of monoclonal gammopathy of uncertain significance (MGUS) after autologous stem cells transplantation in severe autoimmune diseases

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Introduction: Autologous stem cell transplantation (AHSCT) has been increasingly used in the last 15 years for the treatment of severe Autoimmune Diseases (ADs). Despite the efficacy of aHSCT in refractory form of autoimmune disorders, toxicity remains a major issue in this type of non-malignant diseases. Transplant-related mortality and some incidence of long term complications, such as secondary autoimmune disorders [1] or infertility prevent its widespread use. We report here the occurrence of MGUS in patients treated with AHSTC for multiple sclerosis (MS) and systemic sclerosis (SSc)

Materials (or patients) and methods: From 2011 to 2014 14 patients diagnosed with ADs underwent AHSCT in our centre, All patients were mobilized with Cyclophosphamide 4 g/sqm and G-CSF. Thirteen MS patients were then conditioned with BEAM regimen plus ATG, 1 patient without melphalan/ATG. One patient affected by systemic sclerosis was conditioned with Cyclophosphamide 200 mg/kg plus ATG. Each patient underwent routinely follow-up every 3 months up to one year after transplant, and then annually. Follow up visits were comprehensive of clinical examination and blood tests sample to assess immune reconstitution

Results: The median age at transplant was 41 years (range 27-54). Patients were re-infused with a medium number of 7,26x10^6/kg CD 34 + (range 3,06-8,78). The median time to engraftment was 12 days (range 9-15) for PNM and 11 days (range 8-15) for platelets. No TRM was reported. Twelve patients were negative for MGUS at screening tests, whilst 2 were positive, one for IgM kappa and one for IgG lambda, respectively. The median of follow up time was 257 days

(range 31-1180). Eleven patients out of 12 showed the appearance of MGUS after the transplant, and persisted in those with a pre-screening positivity. The median time of gammopathy onset was 92 days (range 38-318). We observed 3 IgG lambda, 4 IgG kappa,1 IgA kappa and 2 IgM kappa, all lower than 1 g/dl. Four MS patients presented a switch of Ig class and 3 (1 SSc and 2 MS) the onset of 2 different component at the same time. MGUS persisted in 3 MS female patients (21%), at 13 months, 40 months and 18 months of follow up respectively. Two of them (14%) were also positive for Bence Jones protein in the urine

Conclusion: A transient MGUS was frequently shown in this small series of patients, early after the transplant. This finding might be related to an immunological unbalance during the immune system recovery. However, in some patients the MGUS persisted beyond one year from HSCT and we strongly suggest a stringent monitoring of serum proteins profile, possibly associated to the storage of serum samples according to international guidelines [2]

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Disclosure of Interest: None declared.

P738

Extracorporeal Photopheresis (ECP) in the Treatment of Secondary Progressive Multiple Sclerosis (SPMS)

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Introduction: The majority of patients with relapsing-remitting MS will eventually evolve into SPMS, with a pattern of gradual and progressive accumulation of disability (Filippi et al., 1997). Mitoxantrone is the only therapy approved for the treatment of SPMS in the United States. However, the efficacy of mitoxantrone in SPMS patients without superimposed relapses is unknown and its chemotherapy-associated toxicity limits its use. Thus, there is an unmet need for the development of new therapies that will slow or arrest disability progression in progressive MS without unacceptable toxicity.

Materials (or patients) and methods: This is a retrospective report of 5 patients treated with ECP at the University of Michigan. All patients had failed more than 3 lines of therapy, including cyclophosphamide and mitoxantrone. We followed the intensive schedule normally used for chronic GVHD patients, and no additional treatments for MS were administered. Patients were adequately consented for the procedure, and IRB regulations were followed. We assessed response to ECP with the MS Functional Composite (MSFC), which includes: 1- Timed 25 ft walk (25fwk), 9 hole peg test for upper extremity dexterity (9hpt), and paced auditory serial addition test (PASAT) for cognition.

Results: Four patients are evaluable for response, and all 5 for safety. Two (Patients 1 and 2) of five heavily pretreated SPMS patients had a significant clinical response, noted in Table 1 as percentage improvement for each component of the MSFC. The response has been durable for both (3+ and 2+years), without requirement of additional therapy. In patient number 2, the response has persisted now for 19 months after discontinuation of ECP). Patient 3 remained completely stable during and after discontinuation of therapy. Patient 4 was not fully evaluable due to severe spasticity, weakness and inability to walk that did not improve with ECP. Patient 5 received a total of 25 treatments, which is early for evaluation of efficacy. All procedures were performed through peripheral access and there were no toxicities or complications other than reversible electrolyte abnormalities.

Table 1

	Treatments N (duration)	*Change in 25fwk*	*Change in 9hpt*	*Change in PASAT*	*MRI*
Patient 1	96 (3y 8mo)	Walker to Cane	+65.4%	+23%	Unchanged
Patient 2	50 (10 mo)	+ 54%	+40%	+25%	Unchanged
Patient 3	53 (12 mo)	Stable	Stable	Stable	Unchanged
Patient 4	39 (7 mo)	NE	NE	Unchanged	Unchanged

Conclusion: ECP is well tolerated and has shown clinical improvement and stabilization of heavily pretreated SPMS in 3/5 patients. Based on these preliminary results, we are currently conducting a single-center, randomized, controlled study comparing ECP to corticosteroids for the treatment of SPMS.

References: Filippi et al. Magnetization transfer ratio changes in a symptomatic lesion of a patient at presentation with possible multiple sclerosis. J Neurol Sci. 1997 Oct 3; 151(1): 79-81.

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P739

Fertility in women after autologous hematopoietic stem cell transplantation (AHSCT) for autoimmune diseases

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Introduction: Autologous Hematopoietic Stem Cell Transplantation (AHSCT) has become successful treatment option showing superiority in randomised clinical trials over the standard treatment in such autoimmune diseases as multiple sclerosis, systemic sclerosis or Crohn's disease. The young women who are consulted prior to this treatment are faced with the absence of data on fertility status after the transplantation and chances of becoming pregnant. The aim of our study was to obtain information on fertility issues from the patients transplanted with hematopoietic stem cells for autoimmune diseases.

Materials (or patients) and methods: An anonymous online questionnaire was distributed among the members of the international web forum of patients after AHSCT for autoimmune disease and the data analysed.

Results: The 28 female patients responded to the questionnaire. Two of them were excluded from analysis due to menopause diagnosed prior to transplant. The most common conditioning regimens used for AHSCT in this population of patients were Cyclophosphamide/Rituximab (27% of patients, $n=7$), Cyclophosphamide alone (23%, $n=6$), BEAM (15%, $n=4$) and Cyclophosphamide/rabbitATG (8%, $n=2$). All patients who were transplanted at 32 years of age or younger restored menstruation after the mean of 4,8 months after AHSCT (Range 1-16). Seventy five % of female patients in the study had children prior to the AHSCT. Fifteen % of all women undergoing AHSCT declared the desire for pregnancy after AHSCT, 25% of those patients became eventually pregnant (which constituted 3,85% of all patients in the study).

Conclusion: Despite relatively small number of patients our online analysis has revealed that the fertility is restored in all patients at 32 years of age or younger regardless conditioning and that many of these women might become eventually pregnant. Moreover, despite the general rates of post-transplant pregnancy remain low at the level of around 4%, it constitutes roughly 25% of women who declare desire for

pregnancy. The patient online forums are useful place for data gathering from patients with rare disease situations, who are scattered between many centres.
Disclosure of Interest: None declared.

P740

Autoimmune lymphoproliferative syndrome (ALPS): Is an allogeneic SCT a therapeutic option? (Case report)

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Introduction: Autoimmune lymphoproliferative syndrome (ALPS) is a T cell immundysregulatory syndrome caused by defective lymphocyte apoptosis via the FAS-mediated pathway. Patients with ALPS usually manifests in early childhood with variable clinical manifestations including lymphadenopathy, hepatosplenomegaly, autoimmunity and increased rates of malignancy. Major diagnostic criteria are chronic non-malignant lymphoproliferation (splenomegaly and/or lymphadenopathy), elevation in peripheral blood double negative T cells (DNT, $CD3^+CD4^-CD8^-$ cells) above 5%, defective in vitro FAS mediated apoptosis and identification of somatic or germ line mutations in the FAS pathway (FAS, FASL, CASP10, NRAS). Minor criteria are autoimmune cytopenias, moderately elevated DNTs and elevated levels of IgG, IL-10, vitamin B12 and FASL. The majority of patients are treatable with immunosuppressive agents. Hematopoietic stem cell transplantation (HSCT) is reported only rarely in the past as therapeutic option.
Materials (or patients) and methods: A16 year old male presented with autoimmune cytopenia (severe neutropenia and thrompocytopenia) with detectable autoantibodies against thrombocytes, cervical and abdominal lymphadenopathy and hepatosplenomegaly. The level of DNTs was initially negative, in consecutive evaluations DNTs were above 5%. He developed several severe septic skin infections, which responded only to prolonged broadspectrum antibiotic therapy. The neutropenia was G-CSF refractory and after detection of compound heterozygote mutations in the FAS pathway (CASP10, CD95/APO-1, FASL, XIAP/BIRC4 and CASP8) the patient underwent HSCT. His family history was negative and he presented with several acquired mutations.
Results: The patient underwent allogenic HSCT with BM from a MUD. The conditioning regimen included ATG (3 x 15 mg/kg), Fludarabine (4 x 40 mg/m^2), Treosulfan (3 x 14 g/kg) and Melphalan (2 x 70 mg/m^2). GvHD prophylaxis consisted of CsA (later tacrolimus), MMF and MTX. The chemotherapy was well tolerated and he achieved a successful engraftment. The $CD34^+$ cells count in the BM was 1,1 x 10^6/kg with leukocyte engraftment on day +34 and the time of neutrophile and platelet recovery was on day +36. He developed a stage III GvHD of the gut which responded very well to steroids and ECP. 38 month after diagnosis and 14 month after HSCT the patient is in very good condition, with no signs of GvHD, no signs of autoimmunity or cytopenia.
Conclusion: This report describes a late onset ALPS increasingly seen due to acquired somatic mutations with a complex compound heterocygosity. The clinical symptoms of ALPS can aggravate substantially requiring HSCT. The use of Treo/Flu based conditioning regimen is in patients with immundysregulatory diseases undergoing HSCT. For now HSCT should be reserved for patients with refractory or severe disease. Inasmuch the secondary acquired malignancies dominate the clinical picture in the future and may influence the indication for SCT is not answered.
Disclosure of Interest: None declared.

P741

Hematopoietic stem cell transplantation for autoimmune diseases in South America

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Introduction: Activity on autologous hematopoietic stem cell transplantation (AHSCT) for autoimmune diseases has begun in South America in 2001. The mainly involved centre at the Ribeirão Preto Medical School, University of São Paulo, Brazil, has been active on the field, especially concerning number of transplanted patients, inovation (type 1 diabetes mellitus) and contributions to the scientific literature. Initially led by Dr. Júlio Voltarelli, the Brazilian Autoimmune Disease Transplantation Program includes five other transplant centres in the country, which also contribute providing patient data and discussing joint protocols.
Materials (or patients) and methods: Transplant centres known to be active on AHSCT for autoimmune diseases were invited to update their reports on number and outcomes for patients treated since 2001. Data were sent on completed Excel sheets and analyzed for descriptive statistics and survival curves.
Results: To date, 241 autoimmune disease patients have been included, 98% ($n = 238$) of those effectively transplanted. Two of the Brazilian centres, the Ribeirão Preto Medical School (FMRP-USP, $n = 205$) and the Hospital Israelita Albert Einstein ($n = 36$) have the largest and longest experience on autologous transplantation in South America. Other four Brazilian centres have also included a total of 8 patients, out of which 6 proceeded to AHSCT. Similarly to the experience reported by international centres, the most frequently transplanted disorder was multiple sclerosis, corresponding to 50% ($n = 121$) of the patients, followed by systemic sclerosis (26%, $n = 63$), type 1 diabetes mellitus (12%, $n = 29$), and systemic lupus erythematosus 3% (7). The remaining 22 patients comprise Takayasu's arteritis (3), neuromyelitis optica (4), polymyositis (1), Behaçet's (2), juvenile arthritis (1) and other neurological diseases (14). For multiple sclerosis, median follow-up after AHSCT was 6.6 (0.5–12) years, event-free survival at 5 years was 50% and transplant-related mortality (TRM) was 2.5%. When patients were stratified by conditioning regimen, TRM was 14% (3/21) for the BEAM + anti-thymocyte globulin (ATG)-treated patients and zero (0/100) for the cyclophosphamide + ATG-treated patients. For systemic sclerosis patients, median follow-up after AHSCT was 3.5 (0.5–10) years, event-free survival at 4 years was 75% and transplant-related mortality (TRM) was 5%. For type 1 diabetes patients, median follow-up was of 5 (1.5-10) years, TRM was zero and five (17%) patients remain completely insulin-free for mean of 4.9 years. Transplants for SLE have the highest TRM. Out of the 11 SLE patients enrolled for AHSCT, 4 (36%) died due to complications during the procedure. Median follow-up for this disease was 4 (1-11) years and disease activity-free survival was 40% in five years.
Conclusion: In conclusion, South America is mostly represented by the Brazilian activity on AHSCT for autoimmune diseases and relevantly contributes to the field in number of transplanted patients. Except for type 1 diabetes mellitus, disease frequency and outcomes are similar to those reported by other international centres.
Disclosure of Interest: None declared.

P742

Allogeneic Haematopoietic Stem Cell Transplantation in the Treatment for Human C1q Deficiency – The Karolinska Experience

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Introduction: Human C1q deficiency is a rare (<100 cases reported) autosomal recessive disorder that is associated with severe systemic lupus erythematosus (SLE) and increased susceptibility to infections by encapsulated bacteria. The lack of C1q has been postulated to result in impaired capacity for clearance of immune complexes and apoptotic cells, and thereby contribute to the development of SLE. Furthermore, the importance of C1q and the classical complement pathway in infections with encapsulated bacteria is well-established. Although the severity of symptoms varies greatly among subjects with C1q deficiency, the onset of SLE is often early in life and the SLE and susceptibility for lethal infections such as meningitis result in increased morbidity and mortality compared to idiopathic SLE. Since C1q is produced by monocytes it has been speculated that allogeneic haematopoietic stem cell transplantation (allo-HSCT) may cure these patients. At our centre, we have so far treated five patients with C1q deficiency. In three cases, SLE symptoms remained relatively mild after the initiation of medical therapy, but two patients developed treatment resistant SLE and we decided to pursue with allo-HSCT.
Materials (or patients) and methods: Here, we report a 9-year-old boy and a 12-year-old girl with refractory SLE due to C1q deficiency, who underwent allo-HSCT. The donors were a matched unrelated female (10/10) and a healthy HLA-identical sibling, respectively. In both donors, C1q production of cultured monocytes, C1q serum levels, and functional properties of the complement classical pathway were confirmed to be normal pre-transplant. A reduced intensity conditioning regimen composed of treosulfan (14 g/m^2) and fludarabine (30 mg/m^2) was started on day -6 and given for 3 and 5 consecutive days, respectively. Thymoglobulin (Genzyme Transplant, Cambridge, MA) was started on day -4 and given daily for four days at a cumulative dose of 8 mg/kg. Graft-versus-host disease (GVHD) prophylaxis was given with cyclosporine and four doses of methotrexate post-transplant.
Results: Both the 9-year-old boy and the 12-year-old girl restored C1q levels as well as the functional properties of the classical complement pathway within one month post-transplant. Simultaneously, the severity of the patients' SLE symptoms was reduced. The boy developed post-transplant lymphoproliferative disease, which resolved after rituximab and donor lymphocyte infusions. Unfortunately, the donor lymphocyte infusions induced cortisone-resistant severe gastrointestinal graft-versus-host disease, and he died from multiple organ failure four months post-transplant. In contrast, the girl is doing well 24 months post-transplant, and clinically all signs of SLE have resolved.
Conclusion: Allo-HSCT can cure SLE in human C1q deficiency. To reduce the risk of transplant related mortality, we propose that transplant should be considered early in subjects resistant to medical therapy.
Disclosure of Interest: None declared.

P743

Long-term follow-up after stem cell transplantation in a child with LRBA deficiency suffering from immunodeficiency, polyautoimmunity and enteropathy

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Introduction: Very early onset of inflammatory bowel disease can occur as a part of a variety of immunodeficiency syndromes in children, causing severe enteropathy unresponsive to standard therapy. A poor prognosis is associated with poor quality of life and a high morbidity from the complications of prolonged immunosuppressive therapy and malabsorption. The IPEX Syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) was described as one of the first monogenetic heritable dysregulatory diseases resulting from mutations affecting regulatory T-cells. However, the genetic defects underlying many of IPEX-like disorders remain unknown. In 2012, mutations in the *LRBA* gene (LPS-responsive beige-like anchor) were described, causing a syndrome of autoimmunity and immunodeficiency.
Materials (or patients) and methods: We report a case of a 12-year-old patient with previously genetically undefined CID. The symptoms started at the age of 6 weeks with an autoimmune enteropathy with lymphofollicular hyperplasia, vitiligo at age of 1, autoimmune hemolytic anemia at the age of 3, polyarthritis, autoimmune thrombocytopenia and neutropenia at the age of 6 years. He required several transfusions and high doses of steroids. Previous immunosuppressive therapy with cyclosporine A, rituximab, azathioprine and methotrexate showed limited response. The immunological work up revealed mild lymphopenia, absent vaccine antibodies and slightly low FoxP3 expression in regulatory T-cells. However, over the course of the disease, severe impairment of T-, B- and NK-cell numbers was observed. He suffered from severe steroid related Cushing syndrome with growth stagnation. SCT was considered as the only curative treatment option despite having no genetically confirmed diagnosis at that stage.
Results: SCT was performed in 2010 using bone marrow from a matched sibling donor after reduced intensitiy conditioning including fludarabin (5x40mg/m^2) – melphalan (1x140mg/m^2), thiotepa (2x5mg/kg) and ATG (3x20mg/kg). GVHD prophylaxis included ciclosporin and low dose methotrexate. No peri-SCT toxicity was observed. Early engraftment and full donor chimerism from day +19 onward. The long term prednisolone treatment was reduced and eventually stopped at day +30. On follow up (5 years), we observed an uneventful clinical course with complete reversal of symptoms, no significant GVHD and growth acceleration.Recently pre-SCT DNA was analyzed by next generation sequencing. Two different heterozygous mutations in the *LRBA* gene, one a novel frame shift mutation in exon 23, and a missense mutation in exon 53, were detected causing LRBA deficiency. While patient's mother carried the frame shift mutation, his father was reported as homozygous for the missense mutation.
Conclusion: This case demonstrates that RIC SCT using Flu/Mel/TT and ATG can cure patients suffering from severe autoimmunity and dysregulative disorders caused by LRBA deficiency. This may provide a major improvement in quality of life at an acceptable risk of toxicity. In order to address the challenging question regarding the optimal conditioning regimen for LRBA deficiency, retrospective analysis of genetically undiagnosed transplanted patients with an IPEX-like disease, might be necessary to provide data on conditioning,

course of the disease and immune reconstitution following SCT in a cohort of LRBA deficient patients.
Disclosure of Interest: None declared.

Chronic leukaemia

P744
Ponatinib for Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ ALL) With History of Stem Cell Transplantation (SCT): Efficacy and Safety Results of the PACE Trial

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Introduction: Ponatinib is a potent BCR-ABL tyrosine kinase inhibitor (TKI) approved for Ph+ leukemia patients resistant or intolerant to dasatinib or nilotinib, or with the T315I mutation. The objective of this retrospective analysis is to describe the efficacy and safety of ponatinib (starting dose 45 mg once daily) in patients with a history of SCT at any time prior to enrollment in the ongoing phase 2 PACE (Ponatinib Ph+ ALL and CML Evaluation) trial (NCT01207440) of ponatinib in patients with refractory Ph+ leukemias.
Materials (or patients) and methods: Forty of 449 PACE patients (9%) had a history of SCT. Patients who had undergone SCT <60 days before the first ponatinib dose, patients with evidence of ongoing graft-versus-host disease (GVHD), and patients who had GVHD requiring immunosuppressive therapy were excluded from study participation.
Results: Of the 40 patients in the PACE trial with a history of SCT, 12 (30%) had chronic-phase CML (CP-CML), 8 (20%) had accelerated-phase CML (AP-CML), 11 (28%) had blast-phase CML (BP-CML), and 9 (23%) had Ph+ ALL at study entry. Seventy-three percent of patients received ≥ 3 TKIs prior to ponatinib treatment, before or after SCT. At ponatinib initiation, median time since diagnosis was 3.8 years and median age was 48.5 years. Twelve patients had the T315I mutation detected at baseline: 1 CP-CML, 2 AP-CML, 5 BP-CML, and 4 Ph+ ALL patients. Twenty-five patients (63%) had an SCT ≤ 2 years (but ≥ 60 days) before starting ponatinib, and 15 patients (38%) had an SCT >5 years before starting ponatinib. Patients may have received intervening therapy between SCT and initiation of ponatinib. With a median follow-up of 12.6 months after ponatinib initiation, median duration of ponatinib exposure was 5.9 months, median dose intensity was 41.8 mg/day, and 45% of the patients had dose reductions. The primary endpoint of major cytogenetic response by 12 months was achieved in 5/12 (42%) CP-CML patients, and the primary endpoint of major hematologic response by 6 months was achieved in 3/8 (38%) AP-CML patients, 4/11 (36%) BP-CML patients, and 1/9 (11%) Ph+ ALL patients. Major molecular response at any time was achieved in 1/12 (8%) CP-CML patients, 1/8 (13%) AP-CML patients, 3/11 (27%) BP-CML patients, and no Ph+ ALL patients. Vascular adverse events were observed in 6/40 (15%) patients: myocardial infarction ($n=2$), pulmonary embolism ($n=2$), angina pectoris ($n=1$), cerebral ischemia ($n=1$), coronary artery disease ($n=1$), portal vein thrombosis ($n=1$), superficial thrombophlebitis ($n=1$), and transient ischemic attack ($n=1$). Additional data regarding the outcome of SCT and other treatments prior to ponatinib will be presented.
Conclusion: Ponatinib treatment induced hematologic, cytogenetic, and molecular responses in a substantial proportion of heavily pretreated CML or Ph+ ALL patients who had a history of SCT. Vascular adverse events were observed in 15% of patients in this analysis. Benefits and risks should be considered when prescribing ponatinib in these patients.
Disclosure of Interest: F. E. Nicolini Personal Interest: Novartis: Advisory boards, consultancy, speaker bureau Bristol Myers Squibb: Advisory boards, consultancy, speaker bureau ARIAD: Advisory board, speakers bureau, D. J. DeAngelo Personal Interest: ARIAD Advisory boards and have received honorarium, E. Abruzzese Personal Interest: Consultant for BMS, Participate at board for BMS, Pfizer, ARIAD and Novartis, J. F. Apperley Personal Interest: Membership on an entity's Board of Directors or advisory committees (ARIAD, BMS, Novartis, Pfizer);, T. L. Holyoake Personal Interest: Financial BMS and Novartis for research funding, BMS, Novartis and ARIAD for consultancy, R. A. Larson Personal Interest: Research support and consulting fees from ARIAD, S. Lustgarten Employee of: ARIAD, Personal Interest: Stock ownership, V. M. Rivera Employee of: ARIAD, Personal Interest: Stock ownership, T. Clackson Employee of: ARIAD, Personal Interest: Stock ownership, M. G. Conlan Employee of: ARIAD, Personal Interest: Stock ownership, F. G. Haluska Employee of: ARIAD, Personal Interest: Stock ownership, M. Talpaz Personal Interest: Research funding (ARIAD, BMS, Sanofi, Incyte, Pfizer), J. E. Cortes Personal Interest: Consultancy (ARIAD, BMS, Pfizer, Teva), research funding (ARIAD, BMS, Novartis, Pfizer, Teva); DWK: research funding (ARIAD).

P745
Outcome of Pediatric Chronic Myeloid Leukemia Diagnosed in Chronic Phase: Single Centre Experience

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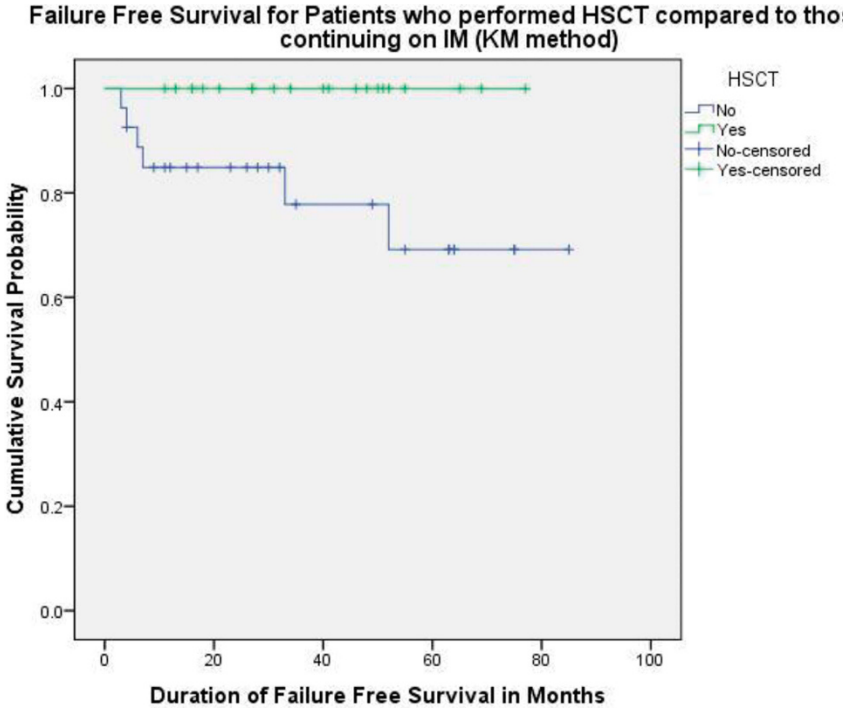
Introduction: Although cure of CML patients is probably only achievable with allogeneic HCT, its treatment-related morbidity and mortality have led to a significant decline in the number of transplants performed in the last 2–3 years and most patients prefer to stay on IM as a more tolerable and effective treatment. The objective of the study is to determine the outcomes in children with chronic myeloid leukemia (CML) diagnosed in chronic phase (CP).
Materials (or patients) and methods: This retrospective study included patients below 18 years old who were diagnosed with chronic phase CML in Children Cancer Hospital Egypt (CCHE 57357) from August 2007 to December 2013 with follow up till October 2014. All patients were initiated on Hydroxyurea ± imatinib mesylate (IM) therapy, and then patients who had available matched related donor (MRD) were subjected to allogeneic HSCT (with Bu/Cy as the conditioning regimen) and patients who had no MRD, continued on IM.
Results: Forty-eight children were analyzed with median age of 13 years (range 20 months– 17 years). Twenty one (44%) of patients proceeded to allogeneic hematopoietic stem cell transplant (HSCT) (all from matched sibling donors, 10 patients received stem cells from peripheral blood and 11 patients received stem cells from bone marrow), while 27 (56%) continued on IM therapy. The median duration of treatment before transplant was 10 months (range 7 to 40 months). From all patients on IM, 1(4%) developed Acceleration and 5 (19%) developed blastic crises at median duration of 6 months after diagnosis. Of the whole transplanted patients, one case showed rising Minimal residual disease (MRD), (Increased level of BCR-Abl transcript measured by quantitative polymerase chain reaction) that received one dose of donor lymphocyte

[P745]



infusion (DLI) and achieved 2nd major molecular response. The 3-Years Failure-free survival (FFS) probability for the transplanted patients was significantly better than those who continued on IM (100% compared to 77.6%, *P* value 0.026). The incidence of transplant related mortality (TRM) at 100 d was 0% and the incidence of chronic graft versus- host disease (GVHD) was 80% in cases with peripheral blood source as compared with 9% in cases with bone marrow stem source (*P* value 0.002).

Conclusion: Considering the lower failure rates and very low TRM associated with HSCT in our pediatric CML patients as compared with IM, our data suggest offering transplant for all patients with available matched related donor. Efforts should be done to closely monitor the response and evaluate causes of failure during TKI therapy.

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P746

Previous second generation tyrosine kinase inhibitors treatment may reduce graft versus host disease incidence in chronic myeloid leukemia patients after allogeneic hematopoietic stem cells transplantation - Polish multicenter preliminary analysis

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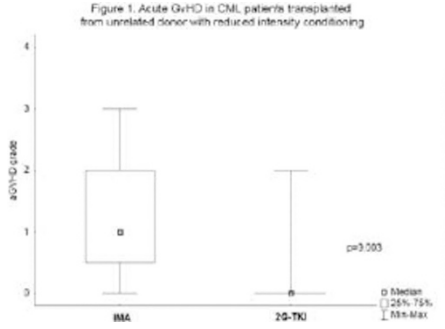
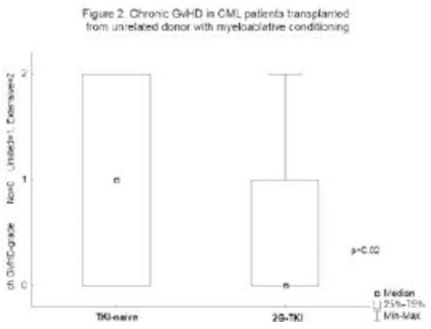
Introduction: It has been 20 years since allogeneic hematopoietic stem cells transplantation (HSCT) became a part of routine treatment of hematologic neoplasms but some complications of the procedure still remains an issue. One of the most serious is graft versus host disease (GvHD). On the other hand some classic indications for allogeneic HSCT which was chronic myeloid leukemia (CML) became "transplantation independent" due to modern and less toxic treatment. Tyrosine kinase inhibitors (TKI) as the treatment of choice for CML are also described as an alternative and adjunctive approach for chronic GvHD especially with fibrotic features. A group of heavily pretreated CML patients still become resistant and they are natural candidates for allogeneic HSCT. In this study we tried to analyze the potential influence of previous TKI treatment in CML patients on GvHD occurrence after allogeneic HSCT.

Materials (or patients) and methods: A cohort of 106 CML patients consisted of three subgroups: TKI naïve ($N=$ 48), treated with Imatinib only (IMA) ($N=30$) and treated with Imatinib and second generation TKI (2G-TKI) (Nilotinib or Dasatinib)($N=28$) was retrospectively analyzed. The median age in groups was 33,31 and 48 respectively ($P=0.02$). Other GvHD related features was as follow: reduced intensity conditioning (RIC) used in 0, 4 (13.3%) and 13 (46.4%) cases ($P=0.04$) and peripheral blood progenitor cells (PBPC) as a transplant material in 18 (37.5%), 14 (46.7%) and 24 (85.7%) cases ($P=0.03$) respectively. There were no significant differences between groups in CD34+ cells number (median dose of CD34+cells/kg was 3.42 (1.0-13.6), 4.6 (1.02-13.5) and 4.0 (1.5-11.2) respectively), number of patients transplanted from unrelated donor (24 (50,0%), 22 (73,3%), 18 (64,3%)), donor-recipient sex and age relation and CMV reactivation ratio. All patients were transplanted from fully HLA matched donors and GvHD prophylaxis was Cyclosporine and Methotrexate based.

Results: GvHD occurrence was analyzed in subgroups as previously described: TKI naïve, IMA and 2G-TKI. Acute GvHD occurred in 20 (41.6%), 12 (40.0%) and 5 (17.8%) cases respectively. There was a significant difference between IMA and 2G-TKI ($P=0.003$) (Figure 1) and TKI-naïve and 2G-TKI groups ($P=0.002$) in unrelated setting with reduced intensity conditioning. Extensive chronic GvHD occurred in 12 (25.0%), 9 (30.0%) and 3 (10.7%) cases respectively. There was a significant difference between IMA and 2G-TKI ($P=0.04$) and

[P746]





TKI-naïve and 2G-TKI groups ($P = 0.02$) (Figure 2) in unrelated setting with myeloablative conditioning. Cumulative probability of overall survival was the highest in 2G-TKI group but the difference was not significant.

Conclusion: Tyrosine kinase inhibitors paths of signaling still remain an issue for research. Our results, if confirmed, probably do not promise to directly impact treatment decisions affecting CML patients but may be a step ahead for better understanding of graft versus host disease biology.

Disclosure of Interest: None declared.

P747

Post-Transplant Strategy For Eradication Of Chronic Lymphocytic Leukemia

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Introduction: Allogeneic peripheral stem cell transplantation (PSCT) may be curative for refractory chronic lymphocytic leukemia. Compared with myeloablative conditioning regimen, a reduced-intensity conditioning regimen tends to decrease treatment-related mortality and increase overall survival after PSCT (Full kimerizm sağlanan hastalarda). The aim of this preliminary study is to evaluate the effectiveness of alternately applied donor lymphocyte infusion (DLI) and rituximab treatment following allogeneic PSCT on the eradication of the disease.

Materials (or patients) and methods: Nine patients with chronic lymphocytic leukemia who were in high risk group according to EBMT criteria underwent allogeneic PSCT were evaluated retrospectively. Median age of the patients was 53 years (range 48-61). Allogeneic PSCT was applied to 6 patients in partial remission and 3 patients in complete remission following FCR, Alemtuzumab or R-bendamustine treatments. Fludarabine/cyclophosphamide/ATG (fludarabine 30 mg/m^2 daily and cyclophosphamide 500 mg/m^2 daily between days -7 and -3, ATG 30 mg/kg daily between days -3 and -1) conditioning regimen was administered. Post-transplantation immunomanipulation (withdrawal of immunosuppression, infusion rituximab, and step-wise DLI) was performed in patients with progressive or residual disease using previously established methods. An escalated rituximab (375 mg/m^{2}intravenously) and DLI (5x10^6/kg T cells) were given at 8-week intervals if there was persistent active disease, or mixed chimerizm.

Results: Neutrophil and platelet engraftment occured at median 12 (9-15) and 12 (10-14) days respectively. Five patients were in partial remission and 4 patients were in complete remission on assessment done on posttransplant month 2. Treatment-related mortality, acute GvHD, severe or unexpected side effects were not observed within the first 100 days after PSCT. The patients were given DLI and rituximab for mean 3 times. Grade I-II cutaneous GvHD developed in 2, grade III cutaneous GvHD developed in 1, grade II pulmonary GvHD in 1, grade II chronic liver GvHD was observed in one patient who were administered rituximab and DLI. Median follow up time was 13 months (4-60). One year survival was 87%. One patient died from the disease at the end of 32 month. Four patients are surviving in complete remission (3 MRD negative, 1 MRD positive), 3 patients in partial remission and one patient has relapsed disease.

Conclusion: We concluded that alternate DLI/rituximab application is effective for achieving full chimerism within 12 months, for disease control and prevention of disease progression and also morbidity and mortality did not increase. Novel transplantation strategies, such as chimerizm ve MRD-based preemptive immune modulation could improve the results and should be tested in the context of clinical trials.

Disclosure of Interest: None declared.

P748

The Outcomes of Allogeneic Transplantation for Myelofibrosis, Single Center Experience

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Introduction: Although new targeted therapy for myelofibrosis has been introduced, allogeneic transplantation still remains the only curative option, especially in the cases where other disease specific mutations than JAK2 or none are detected.

Materials (or patients) and methods: We analysed 63 patients transplanted for primary myelofibrosis or postpolycytemic myelofibrosis between the years 1996 and 2014. Median age of the patients was 55 years, 36 males, 27 females, 14 patients (22%) were $\geq$60 years. The used conditioning regimen was fludarabine and busulfan, the dose of which was 8 mg/kg in reduced intensity conditioning and 16 mg/kg in myeloablative version, further replaced by Busilvex i.v. in myeloablative settings. ATG was used in all unrelated transplants. Peripheral stem cells were used as a graft source, matched siblings 16 (25%) and 47 (75%) unrelated donors.

Results: The engraftment failed in 5 (8%) patients, 2 because of early death. The estimated overall survival in 2 and 5 years was 70%, resp. 65%. 7 patients were retransplanted, 3 because of non-engraftment 4 because of graft rejection. There was no statistical survival difference between patients under age of 60 and above and between myeloablative and reduce intensity conditioning regimens. Lille and IPSS prognostic stratification assessed directly before transplantation tend to statistical significance for the post-transplant survival ($P = 0{,}06$). Surprisingly in our cohort there was significant worse overall survival for sibling donors transplants ($P = 0{,}02$), what needs further evaluation. Splenomegaly more than 10 cm below costal margin tend to be significant for worse overall survival ($P = 0{,}06$), while patients after pre-transplant splenectomy did not differ from patients with normal size spleen.

Conclusion: Similarly to other published data allogeneic transplantation for myelofibrosis is a feasible curative option with relatively good survival results even for patients between 60-65 years.
Disclosure of Interest: None declared.

P749
Relapses over 10 years following RIC conditioning for chronic phase CML

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Introduction: Chronic myeloid leukaemia (CML) was one of the main indication for allogeneic stem cell transplantation before the tyrosine kinase inhibitor (TKI) era. We transplanted 53patients suffered from CML with Dibromomanittol (DBM)/ Cytosine arabinoside (ARA-C)/Cyclopposphamide (CY) conditioning regimen which was a very first reduced intensity conditioning regimen (RIC) known.
Materials (or patients) and methods: As time being we diagnosed five (5/53) patients with very late relapses. All patients were transplanted with their first chronic phase CML from their HLA matched sibling donor after DBM/ARA-C/CY conditioning. One of them had grade 2 acute GVHD, and three of them developed chronic limited GVHD. At the time of relapse they all were free of any immunosuppressive drugs. They were in remission median 18 yeas (11-21 years) with ongoing bcr/abl transcript between of 3-5 logs. Two out of the three women patients became pregnant and delivered healthy new-borns.
Results: Their relapse was detected by chance because stopped visiting their haematologist in the hope of being cured from their CML disease. Four out of five (4/5) patients developed overt hematologic relapse of the CML and one of them presented with granulocytic sarcoma of the sacral region. No molecular biological mutation or additional chromosome aberration were found among the four patients in chronic phase of CML. All patients were commenced on TKI and one received 40 Gray irradiation of the sacral area as well for the granulocytic sarcoma. Three patients achived major molecular response with the restoration of full donor chimerism. One patient has partial (2 logs) response of the bcr/abl transcript. One patients received DLI two times developing limited chronic GVHD and ceased TKI. All the four patients who had overt hematological relapse received bone marrow graft from their brother with no or only limited chronic GVHD (2 no/2 limited chronic).
Conclusion: In summary RIC conditioning and graft versus tumor effect does not cure CML just controls it. Close monitoring of the disease is necessary even deacdes away from the transplant. The proper use interferon alpha and/or DLI are still the best option to enhance graft versus tumour effect. The use of TKI can control effectively the relapsed CML after transplant but not obvious how to use DLI in those cases. It would be important to find markers to monitor the strength of GVL effect
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Disclosure of Interest: None declared.

P750
Evaluation of 272 patients with chronic myeloid leukemia who underwent allogeneic stem cells transplantation between 1998 and 2007

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Introduction: Between 1998 and 2007, the allogeneic stem cell transplantation (ASCT) in patients (pts) with chronic myeloid leukemia (CML), accounted for 32% (272 pts / 838) allografts performed during this period. From January 2008, the availability of Imatib* (cheaper Indian drug previously validated from 2005 to 2007 among pts with CML lacking geno-identical donor), has drastically reduced the indication of the allograft. We report become the long-term (180 months) 272 CML pts after ASCT.
Materials (or patients) and methods: Of these 272 pts whose median age is 33 years (5-57) sex ratio: 1,05. Myeloablative conditioning (MAC) (Tutshka protocol) was applied in 99 pts, median age 23 years (5-45) and a reduced intensity conditioning (fludarabine-busulfan) (RIC) in 173 pts: median age 36 years (18 -57). The pre-transplant status is a first chronic phase in 80% (220/272 pts), the EBMT score of 0-2 in 70% of cases, a positive CMV + donor recipient in 84% of cases. Peripheral blood stem cells (PBSC) in 96% of pts, bone marrow in 9 pts and related cord blood in 2 pts. Median follow-up at December 2014 was 80 months (3-180).
Results: All pts MAC group had aplasia median 14 days (8-75), 89 pts (51%) of the RIC group showed no aplasia and 84 (49%) showed aplasia with a median time of 13 days. Transfusion requirements in the MAC and RIC groups were red blood cells (RBC) 108 and 11 respectively and platelet (CUP) 232 and 20 respectively. Early complications: mucositis grade III and IV in 54% of the MAC group and 9% of the RIC group (p: 0.01) and infection 96% and 11% respectively (p: 10^{-8}). Acute GVHD rates were identical in the 2 groups (48% and 34%) ($P = 0.12$), as well as chronic GVHD (53% and 74%) (p: 0.95). TRM is identical in the 2 groups (36% and 32%) ($P = 0.5$), essentially linked the acute GVHD 14% and 7.5% (p: 0,08) and chronic GVHD 14% and 18% (p:0,08). Relapse was observed in 12 pts (12%) of the MAC group which 10 died and in 18 pts (10%) of the RIC group which 17 died ($P = 0.5$). Late complications related to chronic GVHD responsible for disabling sequelae were observed with the same frequency in the 2 groups. The overall survival (OS) is 60% at 96 months and 55.5% at 180 months, it is identical in the 2 groups (54% in the MAC group and 55.5% in the RIC group) at 180 months ($P = 0.5$).
Conclusion: The results on survival are identical in the two types of conditioning regimen. Early complications and transfusion requirements are less in RIC. The OS of our pts to 8 years is 60%, while the use of Imatinib (Meininger ASH 2008) has produced an OS of 85% led us to the abandonment ASCT in first chronic phase.
Disclosure of Interest: None declared.

P751
Recent results of allogeneic stem cell transplantation for myelofibrosis using reduced intensity conditioning - the Swedish experience

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Introduction: An analysis of the results of allogeneic SCT for myelofibrosis (MF) up to 2009 in the Nordic countries has previously been published (Abelsson et al, Bone Marrow Transpl 47;380, 2012). We have now analysed the outcome in Sweden from 2009 and onwards.

Materials (or patients) and methods: All six allogeneic SCT centres in Sweden reported their data to the Swedish BMT group. The following data were recorded: age, gender, primary/secondary MF, spleen size and DIPSS at transplant, use of ruxolitinib pre-transplant, conditioning, donor source and outcome data.
Results: From Jan 2009 until Sep 2014, 49 patients underwent allogeneic SCT for MF in Sweden. There were 27 males and 22 females. The median age was 60 (25-69) yrs, with 6 patients being <50 and 27 ≥ 60 yrs. Time from MF diagnosis to SCT was median 17 (4-264) months. Nineteen patients had secondary MF (10 after ET, 8 after PCV and 1 after untreated MDS RCMD). Forty patients had enlarged spleen, and 39 had DIPSS high or INT-2. Eight received ruxolitininib pre-transplant. Reduced intensity conditioning (RIC) was used in all patients. The most common conditioning, given to 39 of the patients, was fludarabine 150-180 mg/sqm and busulphan 8 mg/kg PO, or 6,4 mg/kg IV. ATG or alemtuzumab was given to 43 patients. Family donors were used in 15 cases (13 HLA-id sibs, 1 haploidentical and 1 7/8 HLA-matched cousin), and unrelated donors in 34 cases (all but one 8/8 HLA-matched). Stem cell source was PBSC in 46/49 cases.
With a median follow-up of 3 years, survival at 3 years is 64% (69% for patients <60 years and 59% for patients $\geqslant 60$ years) and the event-free survival is 44% (46% for patients <60 years and 43% for patients $\geqslant 60$ years). The cumulative incidence of non-relapse mortality (NRM) at 1 year is 18%, with 6 out of seven events occurring in patients ≥ 60 years. Fourteen patients have either rejected or progressed, all within two years from transplant. Seven of these pts have been re-transplanted with 4 being alive in response. Four out of eight patients pretreated with ruxolitinib either progressed or rejected. In univariate analysis no single factor was significantly associated with survival, but age ≥ 60 yrs and secondary MF had a trend ($P = 0.10$) towards negative prognostic impact.
Conclusion: Compared to previous Nordic results 2000-2009 with RIC for MF, our recent results appear to be equal for younger patients ($\approx 70\%$ survival at 3 years) but slightly better for older patients ($\approx 60\%$ compared to 40% survival at 3 years). Survival for the older patients are mainly hampered by a higher NRM (6/27 patients $\geqslant 60$ years compared to 1/22 patients <60 years), while the efficacy on the disease does not seem to be influenced by age. Relapse and rejection appears a major problem using RIC conditioning. The optimal conditioning which balances between efficacy and NRM remains to be identified both for younger and older MF patients. The aim of the Swedish BMT group is to, together with the MPD group, create more detailed guidelines regarding transplant indication and patient selection, timing of transplantation with respect to pretransplant therapy, conditioning and posttransplant interventions.
Disclosure of Interest: None declared.

P752
Prognostic significance and kinetics of minimal residual disease in chronic lymphocytic leukemia after allogeneic stem cell transplantation

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Introduction: We conducted a retrospective analysis of patients with high risk chronic lymphocytic leukemia (CLL) who have undergone allogeneic hematopoietic stem cell transplantation (allo-SCT) between March 2002 and April 2014 in our centre.
Materials (or patients) and methods: Forty patients (pts) with high risk CLL were identified, who had MRD assessed using allele-specific quantitative RT-PCR at regular intervals after allo-SCT. Patient characteristics were as follows: 24/40 were males, 22 pts received myeloablative/18 reduced intensity conditioning, 34 patients received PBPC and 6 BM grafts. At allo-SCT, 29 pts were chemosensitive, 29 pts were transplanted from an unrelated donor, 12 pts had del 17p-, 16 pts had del 11q- and 12 pts had unmutated IgVH. The median age at allo-SCT was 56 years (range; 42-66), median follow-up (FU) after allo-SCT was 37 months (2-145). At allo-SCT, all pts were MRD positive (MRD pos). 640 samples from patients were analysed for MRD
Results: Overall, 19 (48%) pts died; fourteen (35%) of non-relapse mortality (NRM), five of relapse (13%). Median FU of whole cohort was 37 months (2-145). Estimated 4-years overall survival was 61%, median survival was 65 months.Twenty-two pts reached MRD negativity (MRD neg), at a median of 10 months (range; 1-34); six pts after immune intervention (1x immunosuppression withdrawal, 5x donor lymphocyte infusion (DLI) + rituximab), 11 pts in context of GVHD. Four MRD neg pts died within 6 months (m) after allo-SCT of NRM, 12 pts remained MRD neg (2 out of them died of late NRM), six pts experienced molecular relapse (1 died of NRM at 20 m, 3 are alive at 39, 76 and 100 m, in hematological remission), 2 pts relapsed (1 died of of relapse in extranodal area at 77 m, 1 died of NRM in remission at 82 m). Seven pts reached MRD neg within 12 months after allo-SCT; 1 pt died of NRM (MRD neg), 6 pts live MRD neg. Estimated 4-years relapse rate (RR) was 0% for pts who achieved MRD negativity at 12 m, vs 38% for pts who remained MRD positive ($P = 0.014$). Median follow-up of MRD neg pts was 48 m (4-145). Overall, 18 pts were permanently MRD pos in median FU of 21 m (2-106); six out of them died of NRM, 4 pts died of relapse, 8 pts are alive. Event-free survival was significantly better in pts who reached MRD negativity (61% vs 15% at 4 years, $P = 0.006$), as well as RR (6% vs 68% at 4 years, $P = 0.003$). Seven pts received DLI + rituximab for MRD positivity persisting 6 months after allo-SCT (6 out of them with del 17p-), all are alive in remission, three are MRD negative. In terms of achieving MRD neg, we did not observe significant difference according to type of conditioning, disease status at allo-SCT or cytogenetics (however, 50% of pts with del 17p- recieved preemptive DLI)
Conclusion: Quantitative analysis of MRD is an essential tool for monitoring patients with CLL after allo-SCT. Achieving MRD negativity is clearly associated with long-term disease-free survival. Time to MRD negativity can be relatively long and is probably associated with the induction of GVL activity. It is possible that achieving MRD negativity in patients with del 17p- will be more difficult with the necessity to use immune intervention, but the results are comparable with other patients. The optimal timing and type of immune intervention is to be defined. NRM remains high, mainly due to infections and GVHD.
Disclosure of Interest: None declared.

Haemoglobinopathy and inborn errors of metabolism

P753
Rapid increase of CD8 + T cell count in peripheral blood of pediatric patients after HLA-identical stem cell transplantation for sickle cell anemia

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Introduction: Sickle Cell anemia (SCA) remain a disease with high risk of morbidity and early death, especially in African

patients. Allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative treatment for SCA. To analyze immunoematological reconstitution after transplant, we report our experience concerning HLA-identical HSCT for SCA-patients prepared with the same myeloablative regiment.
Materials (or patients) and methods: This study included 33 consecutive Nigerian SCA patients who underwent bone marrow transplantation from human leukocyte antigen (HLA)-identical sibling donors between 2010 and 2014 following a myeloablative-conditioning regimen. Patients received fludarabine (30 mg/m^2/day) for 5 days and a conditioning regimen including targeted intravenous busulfan (14 mg/kg total dose) and cyclophosphamide (200 mg/kg total dose). To analyse the mechanisms involved in immunological reconstitution post transplant, we analysed T cell subsets by flow cytometry at + 60 post transplant.
Results: All patients had sustained engraftment and remained free of any SCA-related events after transplantation. Sixty days after the transplant, the patients had significantly lower CD4 + T cells in comparison to the controls (15.8 ± 6.9% *vs*. 47.5 ± 6% respectively), whereas CD8 + T cells were the first lymphocytes to repopulate the peripheral blood with up to 45% of these cells being CD8 + T cells (in mean 42.8 ± 15.9% *vs*. 20 ± 7%). All patients displayed reduced numbers of B cells versus normal value, and 20/33 patients had only 0% to 1% of control levels of CD19 + cells. CD3-CD56 + ^{bright} NK cells were 20.7 ± 11.1%, whereas CD3-CD16 + (with cytotoxic functions) were 20.1 ± 11.5%. All patients except one had positive serology for CMV before transplantation. Asymptomatic CMV reactivation occurred in 31 of 33 patients. All patients were provided pre-emptive antiviral therapy and none developed CMV disease.
Conclusion: Our primary finding include the following: 1) rapid increase of lymphocytes in peripheral blood after transplant; 2) rapid expansion of CD8 + T cell but not CD4 T cell counts. Probably reactivation of cytomegalovirus (CMV) infection, observed in 31/13 patients on the early stages of T-lymphocyte recovery after transplant, might induce a dramatic increase in CD8 but not in CD4 T-cell counts.
Disclosure of Interest: None declared.

P754

Successful hematopoietic stem cell transplantation in a patient suffering from pulmonary alveolar proteinosis due to GMCSF-R deficiency allowing Complete respiratory recovery

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Introduction: Granulocyte-macrophage colony–stimulating factor (GMCSF) plays a critical role in alveolar macrophage function and pulmonary surfactant homeostasis. Disruption of GMCSF signaling by mutations of the GM-CSF receptor subunit a or b result (in mice and humans) in pulmonary alveolar proteinosis (PAP). In these conditions, PAP is related to impaired surfactant catabolism in alveolar macrophages. Hematopoietic stem cell transplantation (HSCT) was shown to reverse lung abnormalities in mice with homozygous null mutation of the b sub- unit.
Materials (or patients) and methods: We report the case of a 7 year-old girl born to non-consanguineous parents whose PAP related to *CSF2RA* abnormalities was diagnosed at the age of 2.6 years after several months of progressive insidious tachypnea and hypoxia. Genetic investigation showed complex chromosomes abnormalities with a deletion of both *CSF2RA* alleles. Whole lung lavage was proposed every 6 months during 1.5 years. Despite these treatments, tachypnea, excercice intolerance and hypoxia progressed leading to progressive treatment intensification requiring BAL every 3 weeks and continuous oxygenotherapy from the age of 5.
Results: Given the severe condition of the child and the poor prognosis of this condition, we therefore proposed an HSCT from a 10/10 unrelated donor at the age of 6.3 years old. Respiratory condition at that time showed mild tachypnea at rest, exercise intolerance and continuous oxygen therapy by nasal canular 1-2 L/minute. Pre HSCT lung CT scan showed diffuse bilateral ground-glass opacifications. Pulmonary function tests showed mild restrictive ventilatory defect with residual functional capacity to 80% of normal and decrease of diffusing capacity for carbon monoxide (DLCO) to 65% of normal.
The conditioning regimen consists in IV busulfan 12.8 mg/kg (cumulative AUC 14 312 μM.min), fludarabine 160 mg/m2 and anti-thymocyte globuline 10 mg/kg. The graft contained 7,6 x 10^6 CD34/Kg. Graft versus host disease prophylaxis with ciclosporin from D-1 and mucophenolate mofetil from D0 was given. Aplasia was uneventful and hematologic recovery was achieved on day D15 post HSCT with a 100% donor chimerism (X and Y chromosome FISH analysis on peripheral blood). Respiratory status remained stable during aplasia but mildly progressed concomitantly to haematologic recovery. Bronchoalveolar lavage was than performed on day D15. Chimerism performed on alveolar macrophages was 94% of donor origin. From day 23, respiratory condition rapidly normalised allowing complete and long lasting weaning of oxygeotherapy. Acute skin (grade I) graft versus host disease occurred on day D22 requiring short course steroids. Relaspe skin GVHD (grade II) and gut GVHD (grade IV) occurred on D66 requiring several lines of immunosuppressive drugs. To date, 9 months post-BMT, the child has not presented any recurrence of respiratory symptoms. Respiratory function test have normalized and lung CT scan have returned to normal. A 100% donor chimerism was found to be stable over time. Skin and gut GVHD are currently in remission under immunosuppressive drugs.
Conclusion: We report for the first time that HSCT is a curative treatment for PAP related to *CSF2RA* anomalies in humans. This treatment should be considered in patients severely affected with high dependence to whole lung lavage.
Disclosure of Interest: None declared.

P755

Split Chimerism Between Nucleated and Red Blood Cells in a Beta Thalassemia Patient After Stem Cell Transplantation

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Introduction: Mixed chimerism (coexistence of host- and donor derived cells in the recipient after hematopoietic stem cell transplantation) occurs in approximately ten percent of patients with ß-Thalassemia. Once persistent mixed chimerism (PMC) is achieved patients don't require red blood cell (RBC) transfusion and are accepted as clinically cured despite very low percentage of donor-derived nucleated cells. Because of a patient who remained transfusion independent despite having a long-lasting low donor-derived nucleated cell chimerism (< 10%), we decided to determine donor-recipient origin of red blood cells and erythroid precursors.
Materials (or patients) and methods: A 10 $^{5/12}$ boy with ß-Thalassemia major underwent allogeneic HSCT in 2006 from a 10/10 allele-level matched and blood group matched sibling donor. He conditioned with busulfan and cyclophosphamide and received unmanipulated bone marrow (BM). GVHD prophylaxis consisted of cyclosporine and methotrexate. Peripheral blood and/or BM samples were collected ay days 30, 60 and 180, and therafter as needed for chimerism analyses. Analysis of short tandem repeats was used to determine the donor origin of nucleated cells and burst-forming unit-erythroid (BFU-E) colonies. The donor origin of

RBCs was determined by assessing the effects of hypotonic solutions (0.9%, 0.6%, 0.45% and 0.3% NaCl) on healthy and thalassemic red blood cells with flowcytometry. All samples could withstand hypotonic solutions as low as 0.45%. However, after incubation in 0,3% NaCl, only the microcytic thalassemic RBC in the carrier and the patient survived, whereas donor-derived erythrocytes were all lysed.

Results: The proportions of donor-derived nucleated cells in peripheral blood decreased progressively as follows 99%, 59%, 28%, 16%, 9%, 5% and 8.5% at day 30, 90, 180, 365, 1095, 2190, 2928 respectively. Similar results were obtained from bone marrow-derived nucleated cells and erythoid precursors (6.8% and 4%) on the day of last follow up (day 2928). In contrast the proportion of donor-derived RBCs was 83% indicating the presence of quantitatively different RBC/nucleated cell chimerism. Except for the healthy control (100% of RBC mean fluorescent intensity (MFI):110), all samples contained two separate populations of RBC on flowcytometry; a group with low MFI and another with "normal" MFI: carrier (66% of RBC MFI:67, 34% of RBC MFI: 143), pre-BMT patient sample (26% of RBC MFI:55, 74% of RBC MFI:120) and post-BMT patient sample (17% of RBC MFI:55, 83%of RBC MFI:138). The appearance of separate groups in the post-BMT sample, can be explained by mixed erytrocyte chimerism, whereas the appearance of separate groups in the pre-BMT sample was due to transfusions. The patient remained transfusion independent with no clinical sign of thalassemia. He was accepted clinically cured as supported with the normal pattern of hemoglobin electrophoresis and the normalized reticulocyte count.

Conclusion: Our results showed the presence of a split chimerism between mature erythrocytes and their progenitors with a prominent erythroid engraftment in peripheral blood. This suggests that even a limited amount of normal erythropoietic engraftment might inhibit ineffective erythropoiesis and its clinical outcomes.

Disclosure of Interest: None declared.

P756

Haemopoietic Stem Cell Transplant failure followed by switch to stable and full production of Fetal Haemoglobin: clinical remission despite genetic disease and transplant rejection

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Introduction: High fetal haemoglobin (HbF) levels ameliorate morbidity and mortality in Sickle Cell Anaemia (SCA) and β-thalassaemia. The variability of HbF levels is genetically controlled by multiple genes and recent studies provide new insight into the molecular mechanisms in order to induce the HbF production in adult haemopoietic cells as a promising therapeutic approach to ameliorate the severity of the beta-disorders. A strong support to such novel approaches comes from recent clinical observations carried out by our group

Materials (or patients) and methods: Out of 276 consecutive patients with β-haemoglobin disorders undergoing Bone Marrow Transplant (BMT) at our institution, we observed 4 BMT recipients who developed the reactivation of HbF synthesis after BMT failure and autologous reconstitution

Results: Three patients with β°-thalassaemia major underwent BMT and rejected the first at +40 days, the second one at +90 days and the third one at +18 respectively days after transplant. The autologous recovery was documented (0% residual donor cells) in all cases.

Transfusion therapy was required to support anaemia until +118 and +162 days and +178 days after transplant respectively. Afterwards the Hb levels were steadily over 10.2 g/dl (range 10.2-11.8 gr/dL) without the use of transfusion support and the Hb electrophoresis revealed HbF 99.8% in all 3 cases.

At +93 and +82 and +17 months respectively of ongoing follow-up after graft failure, all 3 patients maintain the sustained and full (99.8%) production of HbF and are transfusion-free. (See the table).

The fourth case was a SCA patient who failed to maintain the engraft of the haplo-identical allogeneic transplant and the autologous recovery was documented. Thirty-two months after the BMT failure, the HbF levels increased to 49.2 and the patient remains with stable Hb level over 11.4 gr/dl and without vaso-occlusive symptoms.

The genetic analysis documented that all patients were carrier of the non-deletion form of hereditary persistence of HbF. The 3 thalassaemic patients exhibit the homozygosity for the –158 (C->T) point mutation in the G γ promoter sequence. (see the table).

Table: Clinical and Haematological parameters in 4 patients pre-transplant and post-BMT failure

Variable	*Case 1*	*Case 2*	*Case 3*	*Case 4*
Sex/Age*	M/18	F/13	M/9	M/5
Disease	β°-Thalassaemia	β°-Thalassaemia	β°-Thalassaemia	Sickle Cell Anaemia
Genetic analysis				
Beta-gene	Homozygo us IVS-1-I	Homozygo us IVS-2-I	Homozygo us IVS II-1	HbS/HbS
Gamma-gene	Homozygous -158	Homozygous -158	Homozygous -158	
Pre BMT Hb (gr/dL)	8.3-9.0	7.0-8.1		7.0-7.4
Post BMT Hb (gr/dL)	11.8-13	10.2-10.55		11.4-12.3
Pre BMT HbF level (%)	73	24		19.3
Post BMT HbF level (%)	99.8	99.8		49.6

Conclusion: Our study showed that the reactivation of HbF synthesis can occur in the adult age and the high levels of HbF provide a therapeutic benefit to the β-disorders. It is likely that the favourable genetic background in these patients concurred the full HbF production.

Disclosure of Interest: None declared.

P757

Hematopoietic stem cell transplantation in patients with neurometabolic diseases: a single center study of unique transplants in Russia

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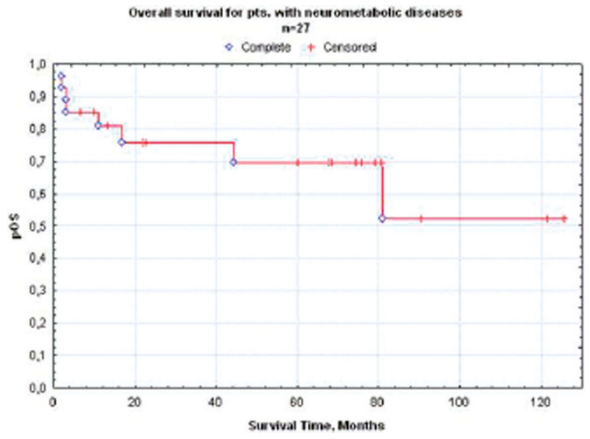
Introduction: HSCT for pts. with neurometabolic diseases is a routine method now. It's very important to provide transplant service for such children.

Materials (or patients) and methods: 27 HSCT were performed during the period 2002-2014. Diagnosis: Hurler syndrome ($n=19$), X-linked adrenoleukodystrophy ($n=4$), metachromatic leukodystrophy ($n=3$), Krabbe disease ($n=1$). MUD HSCT (10/10 and 9/10) were performed in 20 cases, MRD – 7 cases. 9/10 HSCT – 20% ($n=4$), 10/10–80% ($n=16$). Source of stem cells: BM – 74,1% ($n=20$), PBSC – 18,5% ($n=5$), UCB – 7,4% ($n=2$). Age median – 4,14 y.o. Conditioning regimen: Bu/Treo + Flu + Thio/Mel and ATG + Rituximab (in case of MUD HSCT).

Results: All surviving ($n=20$) pts. with complete donor's chimerism ($n=19$) have good response to therapy (best

[P757]



response correlated with short interval to transplant after diagnosis establishing). 1 pt. rejected but stays alive. Reasons of death: infection – 57,1% ($n=4$), GvHD – 28,6% ($n=2$), late progression – 14,3% ($n=1$). Last 5 years experience was characterized by better outcomes (better infection control and GvHD prevention). 3 pts. with X-ALD demonstrated a.GvHD III-IV st. development – only one pt. survived but died later after 7 years after transplant because of disease progression. At a median follow up of 40,53 (7-128 months) the estimated probability of OS was 51,4%, EFS – 49,3%.

Conclusion: Our results suggest that HSCT for pts. with neurometabolic diseases is an effective way to stop neurodegenerative processes in case of successful transplant outcome. Results of HSCT become better due to improvement of infection and GvHD control. X-ALD pts. require additional attention to GvHD prevention.

Disclosure of Interest: None declared.

P758

Safety and efficacy of deferasirox in beta-thalassemia major patients after hematopoietic stem cell transplantation: Baseline data of a phase II, multi-center, single-arm, prospective study

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Introduction: Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for thalassemia major (TM) patients. Although patients usually become transfusion independent after HSCT, they continue to suffer from iron overload (IOL) for years. It is important to control IOL in post-SCT setting. This could be achieved using either deferoxamine or phlebotomy which is poorly tolerated. Recipients transplanted from a thalassemia trait donor are also poor candidates for phlebotomy due to lower hemoglobin levels. Therefore it is significant to investigate other methods of iron removal. To our knowledge this is the first prospective randomized trial evaluates the impact of deferasirox (DFX) on outcomes of patients with IOL after HSCT in Turkey.

Table 1.

Characteristics	DFX (N=26)
Mean age ± SD, years	8.9±3.8
Male: female, n	18:8
Mean hemoglobin ± SD, gl/dL	11.5±1.7
Median serum ferritin (range), ng/mL	1701 (934-3009)
Mean LIC ± mg Fe/g dw	12.2 (2.8-43)
Mean cardiac T2*, ms	26.4 (4.4-41)
Mean ALT ± SD, IU/L	42.7±31.8
Mean creatinine ± SD, mg/dL	0.4±0.1

Here were report on the screening status of the patients who went into the DFX-treatment phase of the study.

The objective of this Novartis sponsored study is to determine the safety and efficacy of 52 weeks of DFX therapy in the treatment of IOL during the post-transplant period in patients with TM prospectively. We aimed to present baseline characteristics of patients who enrolled to the study.

Materials (or patients) and methods: The study was conducted in six centers from Turkey between October 2013-2014. Patients were enrolled at least 6 months after HSCT and 3 months after cyclosporine cessation. Any contraindication for DFX, transfusion dependency, severe complications of HSCT (e.g. GVHD) and 2 years subsequent to HSCT were main exclusion criteria. IOL was defined as SF level of $>1000\,\mu g/L$ or cardiac MRI of ≤ 20ms or LIC of $>5\,mg/g$ dw. SF evaluations were planned every 28 days. Liver (R2 MRI) and cardiac MRI (T2*) were performed at screening and final visits. Patients were assigned to DFX 10 mg/kg daily. Dose adjustments were allowed in step of 5 mg/kg/day gradually up to 20 mg/kg/day at the discretion of the investigator.

Results: Patient enrollment completed with 26 patients. Almost one third of the patients were female (31.0%) and the median age was 8.5 years (3.5-16.7). The median time since HSCT was 14 (7-24) months. 18 recipients transplanted from a thalassemia trait donor. Majority of patients (81%) had SF between 1000-2500 μg/L and LIC between 5-15 mg/g dw (65%). Severe cardiac IOL (T2* 5-10ms) was determined in only one patient with moderate LIC and SF between 1000-2500 μg/L. Six patients completed study as of December. Baseline characteristics of the patients are summarized in Table 1. An interim analysis on effectiveness and safety will be presented subsequently.

Conclusion: Discussion and Conclusion: Since several trials indicate that IOL was associated with negative impact on outcome after SCT, iron chelation therapy (ICT) prior to HSCT is standard of care. In this study the discordance between SF levels and higher LIC can be a result of late pre-transplant intensive ICT. It is important to note that the ideal situation would be continuous and regular lifelong ICT achieving negative iron balance, rather than intensive pre-transplant regimen as it was recommended by TIF. Baseline characteristics in this study suggest that IOL persist for years after transplantation. Future results of this prospective trial on usage of DFX at post-HSCT would be useful to better understand the efficacy and safety of DFX for pediatric population.

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P759
Abstract Withdrawn

P760
The first case of secondary polycythemia due to iron chelation therapy

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Introduction: We report the first case of secondary polycythemia (SP) due to iron chelation therapy (ICT) in a patient suffering contemporary of liver chronic graft-versus-host-disease (cGVHD) and chronic liver disease (CLD) caused by iron overload (IO).
Materials (or patients) and methods: A patient with high-risk acute myeloid leukemia underwent allogeneic haematopoietic progenitor cell transplantation from a matched related donor after reduced intensity conditioning. GVHD prophylaxis was cyclosporine-A (CyA)-based. No complication occurred until tapering of CyA when an increase of liver enzymes was documented. Liver biopsy showed CLD correlated to hepatic hemosiderosis and GVHD. Serum ferritin levels (SFL) were 4840 ng/mL. The patient started subcutaneous desferrioxamine (DSF) 40 mg/kg and prednisone plus CyA according to Seattle protocol with normalization of liver exams and reduction of IO (after 6 months, SFL were 726 ng/mL). During these months, hematocrit (Hct) reached values up to 55%. We excluded all known cases of SP and the patient underwent phlebotomy until Hct normalization. In the suspicion of erythropoiesis (EP) stimulation by DSF, we suspended ICT with stable date of SFL of 700 ng/mL, normal liver function, bone marrow (BM) morphology and Hct. After 4 months, we rechallenged the patient to DSF after collecting serial (time 0) of serum, peripheral blood and BM. Clonogenic tests were performed in the absence and in the presence of saturating dosage of erythropoietin (EPO). We performed gene-expression assay (GEP) of genes involved in erythroid lineage differentiation and regulator microRNAs (miRNAs). After a month (time 1), we had to interrupt it because a new increase of Hct with need of phlebotomy. The drug was no longer taken and Hct values remained normal until last followup (time 2).
Results: Our case is the first report of improvement of EP during ICT until reaching SP. From the clinical side, ICT was proved as responsible for SP by the same effect on Hct during two spaced treatments. On the biological side, DSF improved EP modifying GEP of specific genes and miRNAs subsets involved in EP. GATA1-2 gene transcription orchestrates erythroid differentiation and in our patient, during ICT, GATA1-2 mRNAs showed a significant increase. The increase of expression levels was detectable for RUNX1, HIF1a, LMO2 and the changes of miRNA expression were exactly as reported for erythroid progenitor recruitment and commitment. These results suggested that down-modulation of 146,150,155,223-miRNAs depended on the drug. Specifically, the first RUNX1-target miRNA (miR-223) was down-modulated at time 1,2. Its decline was inversely correlated with the increase of mRNA targets (RUNX1, LMO2). Besides, miR-146 and miR-155 were down-regulated after 6 months of DSF wash-out. It's known that EPO acts as proliferation/survival factor of erythroid lineage-committed cells. At time 0,1,2 serum endogenous EPO levels were never above the normal values suggesting that the increase of EP was not dependent on EPO. In contrast, results of clonogenic tests showed that the precence of exogenous EPO induced growth of erythroid colonies significantly higher during time 1 versus time 0 and 2.
Conclusion: we think that this case of SP due to ICT confirms the effect on EP by DSF, clarifying some biological mechanisms. Other studies are necessary to confirm these data
Disclosure of Interest: None declared.