

## PHYSICIANS ABSTRACTS

**O001**

### **Multiple inhibitory receptors are expressed on central memory and memory stem T cells infiltrating the bone marrow of AML patients relapsing after allo-HSCT**

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**Introduction:** In patients with acute myeloid leukemia (AML), relapse is the major cause of death after allogeneic HSCT.

**Material (or patients) and methods:** To investigate if T-cell dysfunction is associated to post-transplant relapse, we longitudinally analyzed T-cell dynamics in bone marrow (BM) and peripheral blood (PB) of 26 AML pts receiving HSCT from HLA identical (HLAid, 14 pts) or HLA haploidentical (haplo, 12 pts) donors. Samples were analysed by multi-parametric flow cytometry to investigate the expression of inhibitory receptors (IRs) on CD4 and CD8 T-cell subsets defined by CD45RA, CD62L and CD95 expression. The proportion of regulatory T cells (Tregs; CD4+/CD25+/FoxP3+) was also assessed.

**Results:** BM and PB were collected 60 days after HSCT and at the time of relapse (median 219d; 14 pts) or, if complete remission (CR; 12 pts) was maintained, at 1 year. Samples from 8 healthy donors (HD) were used as controls. After transplant, both BM and PB T cells showed a preferential late differentiation profile ( $P < 0.05$ ) when compared to HD. BM and PB T cells from CR pts displayed a lower CD4/CD8 ratio compared to HD, at both time points ( $P < 0.001$ ). A higher proportion of BM Tregs was documented at relapse ( $P < 0.01$ ), independently from the donor source. We next investigated the expression of several IRs as T-cell exhaustion markers. After haplo-HSCT, PD-1, CTLA-4, 2B4 and Tim-3 were significantly upregulated in BM and PB T cells at all time-points, compared to HD and independently from the clinical outcome. Conversely, after HLAid-HSCT, at the late time-point, patients who relapsed, displayed a higher frequency of BM infiltrating T cells expressing PD-1, CTLA-4 and 2B4 than CR patients and HD ( $P < 0.05$ ). We then investigated the profile of each T-cell subset in our cohorts of patients. In the BM of HD the expression of IRs was confined to effector memory and effectors. While a similar IR distribution was observed in CR pts, at relapse, PD-1, 2B4 and Tim-3 were also significantly upregulated in BM infiltrating central memory and memory stem T cells. Interestingly, at relapse, leukemias expressed PD-L1 (8/8 cases) and Galectin-9 (5/8). A linear regression analysis revealed a significant association between the levels of Tim-3 on BM CD8 cells and that of Galectin-9 on autologous AML blasts, suggesting a preferential role for this immunomodulatory axis in the transplantation context. Finally, results were also analyzed with the bh-SNE algorithm, an unbiased

computational method that distributes single cells in bi-dimensional maps according to the fluorescence intensity. Based on phenotype similarities, the algorithm positioned HD samples separately from transplanted pts and HLAid separately from haplo-pts. 93 significant clusters were identified. Clusters associated with relapse after HLA-id (5) and after haplo (15) were composed of CD8 and CD4 T cells expressing preferentially multiple IRs, while CR-specific clusters were diminished in IR fluorescence.

**Conclusion:** After HSCT, the molecular signature of exhausted CD8 cells in relapsing pts includes PD-1, CTLA-4, 2B4 and Tim-3. The expression of IRs on early differentiated central memory and memory stem T cells at relapse suggests a wide immunological dysfunction mediated by AML relapsing blasts.

**Disclosure of Interest:** Luca Vago, Fabio Ciceri and Chiara Bonini equally contributed to the abstract. None declared.

**O002**

### **Tissue instruction dictates spatial diversity of effector T cell programs in graft-versus-host disease**

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**Introduction:** It is not known why only certain tissues are prone to graft-versus-host disease (GVHD) injury following allogeneic hematopoietic stem cell transplantation (allo-SCT) despite widespread antigen expression. Although it is accepted that disruption of specific effector molecules can have distinct effects upon individual GVHD organs, there has been no unbiased or systems-wide approach to defining the mechanisms underlying tissue-specific pathology. We have adopted a systems immunology approach in mice and humans to address the hypothesis that GVHD target tissues exert dominant, idiosyncratic roles in regulating effector T cell (Teff) functions.

**Material (or patients) and methods:** In murine models of GVHD, we tracked the evolution of the donor Teff transcriptomes at multiple sites and time points within the same host following allo-SCT. Donor derived CD8+ T cells were flow sorted from multiple organs in mice developing GVHD: Model 1 – transfer of B6 polyclonal T cells to MHC-matched, multiple minor antigen-mismatched 129 BMT recipients; Model 2 – transfer of monoclonal HY-specific TCR-transgenic MataHari CD8+ T cells to MHC matched, B6 male BMT recipients.

**Results:** Principal Component Analysis revealed that (1) transcriptomes of CD8+ Teff cells in peripheral tissues and lymphoid organs were very distinct, (2) these profiles diverged sharply between the different GVHD target organs, and (3) also segregated within individual sub-compartments of single organs. Weighted Gene Correlation Network Analysis showed that T cell programs in GVHD exhibited a modular structure with separable programs underpinning priming and initial tissue homing versus tissue occupancy and injury, independent of the TCR repertoire and antigen distribution.

Consistent with the concept of compartment-specific interactions driving Teff cell programs, we found that dermal-epidermal migration of Teff was linked to up-regulation of effector programs related to cell cycle commitment, mRNA synthesis and metabolic activity. To investigate the mechanisms imposing skin-specific regulation of Teff cell functions, we evaluated changes in the transcriptome and function of CD8+ T cell in the presence or absence of Langerhans cells (LC). In the absence of LC, T cells were incapable of up-regulating the full panoply of effector genes, showed impaired differentiation into resident memory cells and failed to induce cutaneous GVHD, an effect that required in situ interaction between LC and T cells within the epidermis. We identified a feed-forward loop requiring LC responsiveness to IFN- $\gamma$  that enabled LC to induce cognate activation and expansion of incoming T cells, providing Notch-dependent signals to enhance local cytokine production by Teff and Notch-independent signals to promote resistance to apoptosis.

**Conclusion:** Collectively, these data demonstrate that GVHD is defined by tissue-autonomous instruction of Teff cell identity; in the skin, this is dictated by migration to the epidermis and interaction with LC in situ. Our work provides a rationale for precision therapies directed at blocking GVHD-inducing programs in individual tissues. By avoiding global immune suppression, these approaches may permit preservation of graft-versus-tumour effects following allo-SCT.

**Disclosure of Interest:** None declared.

### O003

#### Unmanipulated haploidentical stem cell transplantation in adults with Acute Lymphoblastic Leukemia: a study on behalf of the Acute Leukemia Working Party of the EBMT

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**Introduction:** Allogenic hematopoietic stem cell transplantation (SCT) has been increasingly used for treatment of adult acute lymphoblastic leukemia (ALL). For patients lacking an HLA-matched related or unrelated donor, unmanipulated haploidentical (haplo)-SCT is a potential alternative, however its value in ALL setting has not been evaluated so far.

We aim to analyze results of unmanipulated haplo-SCT for adults with ALL and to identify prognostic factors.

**Material (or patients) and methods:** We performed a retrospective analysis on 208 adult patients transplanted in EBMT centres from 2007 to 2014. The main endpoints were to

assess overall survival (OS), leukemia free survival (LFS), relapse incidence (RI), non-relapse mortality (NRM), and GVHD and Relapse free survival (GRFS).

#### Results:

Median age at haplo-SCT was 32 years (18-76) and the median year of transplant 2012 (2007-2014). Disease status at transplant was: first complete remission (CR1) for 90 (44%), second CR or subsequent (CR>1) for 59 (28%) and advanced for 59 patients (28%). Among 125 patients with available information on cytogenetics, 48 (38%) were Philadelphia positive. Immunophenotype was T-cell for 33% of patients. Median follow-up of was 16 months (range 1-67).

Stem cell source was bone marrow (BM) for 91 (44%) patients, and peripheral blood (PB) for 117 (56%). Myeloablative conditioning (MAC) was used in 149 patients (72%) and reduced intensity regimen (RIC) in 59 patients (28%). GVHD prevention strategy was based on either post-transplant Cyclophosphamide (PT-Cy) for 132 (52%) patients or *in vivo* T-cell depletion with ATG for 124 patients (48%). Cumulative incidence (CI) of neutrophil engraftment at 60 days was 93%. CI of acute GVHD grade II to IV was 31%, grade III-IV 11% and chronic GVHD 27%. The 2 years CI of non NRM was 26% for patients transplanted in CR1, 36% in CR>1 and 35% in active disease ( $P=0.27$ ). Respective RI according to disease status was 26%, 32%, and 60%. Probability of OS at two years was 49% in CR1, 32% in CR>1 and 11% in advanced disease ( $P<0.001$ ), while LFS was 45%, 32% and 5%, respectively ( $P<0.001$ ). The GRFS at two years was 39% in CR1, 22% in CR>1 and 5% in advanced disease ( $P<0.001$ ).

In a multivariate model restricted to patients in CR, factors associated with decreased NRM were: Pt-Cy vs ATG (HR=0.47, 95%CI 0.25-0.90,  $P=0.02$ ) and Karnofsky performance status (KPS)>90 (HR=0.29, 95%CI 0.15-0.57,  $P=0.0003$ ). No independent risk factors were found to be associated with RI. Use of PT-Cy vs ATG was independently associated with better LFS (HR=0.62, 95%CI 0.39-0.99,  $P=0.047$ ) as well as disease status at transplantation (CR vs advanced stage) (HR=0.59, 95%CI 0.36-0.94,  $P=0.03$ ). KPS>90 (HR=0.48, 95%CI 0.29-0.79,  $P=0.004$ ) and use of BM graft were associated with increased GRFS (HR=0.61, 95%CI 0.39-0.96  $P=0.03$ ).

**Conclusion:** Unmanipulated haplo-SCT is a valid option for adult patients with ALL. 49% OS and 45% LFS at 2 years post transplantation in first CR and impressive 39% GRFS.

Disease status at SCT, performance status and the type of GVHD prevention were the main factors affecting outcomes. The use of PT-Cy should be considered a preferable strategy in this setting. Novel interventions to decrease transplant related toxicity and relapse are in need in order to further improve outcomes.

**Disclosure of Interest:** None declared.

### O004

#### The Inborn Errors Working Party study of long-term outcomes of haematopoietic stem cell transplantation in patients with severe combined immunodeficiencies

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**Introduction:** SCID are a group of disorders leading to T-, +/-B- and NK- cell maturation and function defects presenting with life-threatening infections in infancy and early childhood. The only curative therapy for many years was HSCT,

but there are few data on long-term outcomes in immune function, physical status and quality of life in SCID patients after HSCT.

**Material (or patients) and methods:** 183 patients with SCID (using PIDTC SCID definitions,<sup>1</sup> and including reticular dysgenesis) were transplanted more than 20 years ago in Europe. M/F ratio was 136/47. Median age at diagnosis was 0.2 months (range 0-12), at transplant 6.1 months (range 0.4-13.7). 53 patients had CgC, 17 - ADA, 16 - JAK3, 14 - Artemis, 8 - RAG 1 and 2, 8- AK2, 6 -IL7Ra deficiencies, 2 - other known and 59 - unknown defects. 68 patients received conditioning (52 had significant pre-transplant infections), 115 - had unconditioned HSCTs. 126 had haploidentical (8 - undepleted), 42 - matched related, 9 -mismatched related, 6 -unrelated donors.

**Results:** The median of last FU was 23,28 years (10.4-42.7). Overall survival was 0,58 (95% CI 0.5-0.66). Event free survival (deaths, re-transplant considered as events) was 0.46 (95% CI 0.34-0.57). There were no significant difference in OS ( $P=0.26$ ) and EFS ( $P=0.04$ ) in conditioned and unconditioned transplants. 40 patients required second (11 after unconditioned and 29 after conditioned transplants) and 7 third transplants. 69 patients died early (before 5 years) after transplant (57 had infections before transplant), 4 patients died late after first transplant but early after 2 and 3 HSCTs. Transplant related mortality was 0,32 (95% CI 0.26-0.4) (no difference in conditioned and unconditioned transplants,  $P=0.46$ ) 49 patients (26.7%) had acute GVHD grade 2-4. 26 patients (14.2%) had chronic GVHD.

60 patients were alive with more than 20 years follow up. 11 patients had impaired immune function (8 after conditioned and 3 after unconditioned transplants) with low lymphocyte levels or TRECs or required IVIG and antimicrobial prophylaxis. 19 patients had persistent warts (CgC - 7, JAK3 - 5 as previously reported<sup>2</sup>, but also 2 ADA, 1 RAG2, 1 Artemis, 1 IL7Ra, 1 T-B+NK- and T-B- phenotypes). One patient developed malignancy (meningioma). 4 patients had autoimmunity (SLE, hemolytic anemia, vitiligo, autoimmune hepatitis).

7 patient's quality of life considered as below normal, 17 patients had university/college education (for 10 - unknown).

**Conclusion:** Importantly, the study results show no significant difference in OS, EFS and TRM in patients after conditioned and unconditioned HSCTs. One of the most important problem of the study is missing follow up data. However, preliminary analysis demonstrates the need of further estimate of risk factors for impaired immune function, physical status and quality of life in SCID patients long-term after HSCT.

**References:** 1. J Allergy Clin Immunol 2014;133:1092.

2. Laffort C et al. Lancet. 2004;363:2051

**Disclosure of Interest:** None declared.

## O005

### Pre-emptive il-15-activated cytokine-induced killer cell infusions in high-risk leukemia patients after allogeneic stem cell transplantation to treat minimal residual disease

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**Introduction:** In high-risk leukemia patients the major causes for post-transplant mortality are relapse and treatment related toxicity. Novel specific/targeted cellular therapies generated to maximize anti-leukemic capacity while limiting toxicities are currently being tested in early phase clinical trials. CIK cells may represent a technically much more simple and clinically

almost universally efficacious alternative to targeted cell-based anti-cancer treatments such as CAR-armed T lymphocytes. We present the results of a pilot trial using *in vitro* generated IL-15 activated CIK cells.

**Material (or patients) and methods:** Between September 2012 and November 2015, leukemia patients who showed chemotherapy-resistant disease at the time of HSCT or evidence of molecular relapse (mixed chimerism (MC)  $\geq 1\%$  of recipient (autologous) signals; detectable MRD,  $\geq 10^{-6}$  or BCR-ABL/ABL  $\geq 10^{-4}$ ) were included in this study. CIK cells are a licensed cellular therapy for patients with high-risk leukemia and myelodysplastic syndrome in Germany (ATMP § 4b Abs. 3 AMG, license number PEI.A.11630.01.1) and the authors hold a manufacturing license and domestic marketing authorization for CIK cells. CIK cells were manufactured and cleared as described and dispensed by the qualified person of German Red Cross Blood Service. The content of CD3<sup>+</sup>CD56<sup>+</sup> CIK cells in the CIK product was measured at the end of the manufacturing process and defined the maximally allowed total cell dose. CIK cell treatment was initiated after written informed consent. Altogether 18 pediatric and 2 adult patients with high-risk leukemia (ALL,  $n=13$ ; AML,  $n=6$ ; CML,  $n=1$ ) after allogeneic stem cell transplantation received IL-15-activated CIK cells. CIK cells were generated from peripheral blood mononuclear cells of the original stem cell donors (HLA-matched,  $n=8$ ; haploidentical,  $n=12$ ). 12 out of 20 patients (60%) were treated upon molecular relapse, 8 out of 12 patients (40%) with evidence of pre-transplantation refractory disease were treated.

**Results:** In total, 61 infusions were administered, containing escalating doses of CD3<sup>+</sup>CD56<sup>+</sup> T cells and an interval of 4 to 8 weeks between infusions. Median CD3<sup>+</sup>CD56<sup>+</sup> T cell numbers infused were 37.6 (1-100)  $\times 10^6$ /kg within a total of 33 matched and 5.9 (1-32)  $\times 10^6$ /kg within a total of 28 haploidentical infusions. Acute graft versus host (aGVHD) <sup>o</sup>1 was observed in 3 out of 20 patients (15%), whereas another 3 patients (15%) developed <sup>o</sup>3 aGVHD after haploidentical CIK cell treatment. Three/20 patients (15%) died of post-transplant complications apparently unrelated to CIK cell treatment. 9/12 patients (75%) with molecular disease responded to CIK cell treatment, of whom 6 (50%) showed sustained clearance of MRD. Complete molecular remission was maintained in another 7 out of 8 patients with prophylactic treatment (88%). Altogether, at a median follow up of 4.6 months (range, 0.6-38.7 months) since first CIK cell treatment 13/20 patients (65%) remained in complete molecular remission, 7/20 patients (35%) experienced hematological relapse, of which 3 could be rescued by a second transplantation.

**Conclusion:** In conclusion, this pilot trial confirms feasibility, safety and clinical activity of CIK cell treatment in high-risk leukemia after HSCT and thus provides a strong rationale for the up-coming prospective multicenter trial.

**Disclosure of Interest:** None declared.

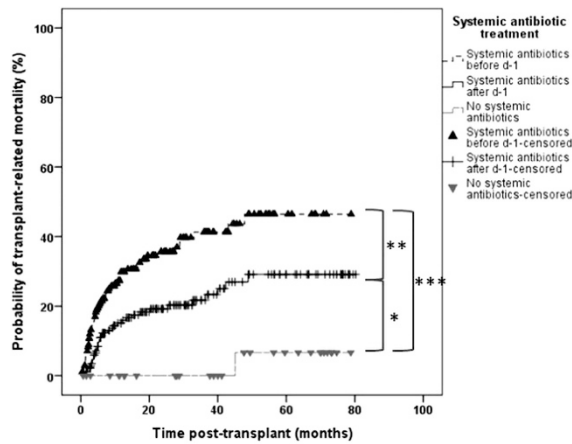
## O006

### Early systemic broad spectrum antibiotic treatment increases risk of graft versus host disease and treatment-related mortality after allogeneic stem cell transplantation - possible role of indirect effects by microbiome disruption

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**Introduction:** Allogeneic stem cell transplantation (ASCT) is a curative treatment option for a variety of hematopoietic disorders. However, ASCT carries a high risk of infectious complications that require the use of broad-spectrum



\* 0.013; \*\* 0.001; \*\*\* <0.001

antibiotics that combat not only pathogenic but also commensal bacteria. There is increasing evidence, that loss of commensal microbes, in particular species of Clostridial Cluster XIVa, in the gut at the time of ASCT is associated with a higher risk of acute gastrointestinal Graft versus Host Disease (GvHD) and poor outcome [1].

**Material (or patients) and methods:** Here we conducted a retrospective analysis of 626 patients undergoing ASCT at two centers (Regensburg/New York) regarding risk factors for additional early systemic antibiotic treatment and regarding the impact of timing of antibiotic treatment on the incidence of acute GvHD, transplant-related mortality (TRM) and intestinal microbiome composition.

**Results:** In the first center (Regensburg, 380 patients) we identified in multivariate analysis early neutropenia ( $P < 0.001$ ) as major risk factor for early antibiotic treatment. Start of systemic antibiotic treatment prior to ASCT correlated with severe GvHD (grade II-IV,  $P=0.03$ ). Early systemic antibiotic was associated with higher TRM (34.1%, 58/170) compared to later administration of systemic antibiotics (19.7%, 35/178,  $P=0.001$ ) or no antibiotic at all (3.1%, 1/32,  $P < 0.001$ , Figure 1). Timing of antibiotic treatment was the dominant independent risk factor for TRM in multivariate analysis. The highest TRM (53%) was observed in patients receiving both prophylactic and therapeutic antibiotics active against commensal anaerobic bacteria ( $P=0.03$ ). Early antibiotic treatment was further associated with lower urinary 3-indoxylsulfate levels ( $P < 0.001$ ) and decreased abundance of members of Clostridial Cluster XIVa ( $P=0.002$ ) on and in the days following ASCT. The main results were validated in the second center (New York, 246 patients) using a different approach of antibiotic prophylaxis. Independently of antibiotic prophylaxis, early additional systemic antibiotic again was associated with higher TRM compared to later administration of systemic antibiotics ( $P=0.002$ ) or no antibiotic at all ( $P=0.004$ ). Regarding antimicrobial disruption clostridial abundance was higher in the late AB group than in the early AB group ( $P=0.04$ ).

**Conclusion:** Our data suggest that at least early administration of systemic antibiotics with activity against commensal clostridial species have a negative impact on outcome and that antibiotic strategies should be reassessed to avoid major microbiota disruption prior to ASCT.

**References:** 1. Weber, D., et al., Low urinary indoxyl sulfate levels early after ASCT reflect a disrupted microbiome and are associated with poor outcome. *Blood*, 2015.

**Disclosure of Interest:** None declared.

## O007

### Infusion of donor T cells transduced with inducible Caspase 9 (BPX-501 cells) is a safe and effective strategy to accelerate immune recovery in patients with non-malignant disorders after T cell depleted haplo-HSCT

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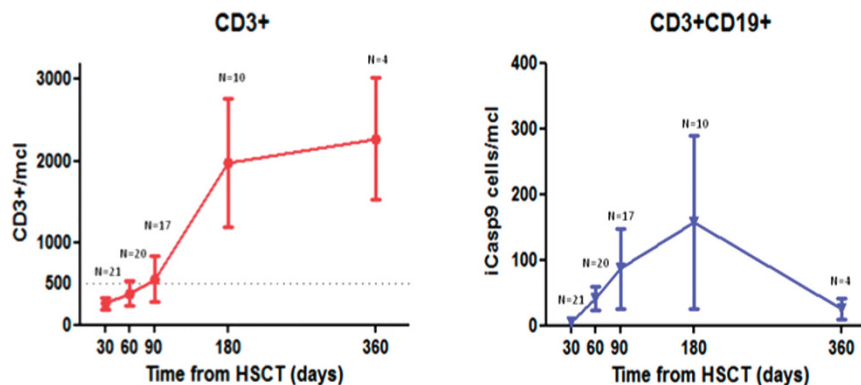
**Introduction:** T-cell depleted allo-HSCT represents a valuable option for those patients (pts) affected by a non-malignant disorder potentially benefiting from an allograft and lacking a suitable HLA-matched donor. We recently reported on 33 children with various non-malignant diseases (hist. cts.) who were given an  $\alpha\beta$  T-cell/B-cell depleted allo-HSCT from a parent, showing a 2-year probability of disease-free survival of 90%, with 3 pts dying of viral infections (Bertaina, et al. *Blood* 2014 and updated unpublished results). Through this approach, the recovery of innate immunity was fast, while reconstitution of  $\alpha\beta$  T cells in the first 2-3 months after transplantation was suboptimal (Airoldi et al *Blood* 2015). In order to accelerate the recovery of adaptive immunity, we designed a phase I/II trial aimed at testing the safety and efficacy of post-transplant infusion of donor-derived T cells transduced with the new iC9 suicide gene (BPX-501 cells, Di Stasi et al. *N Engl J Med* 2011, Zhou et al. *Blood* 2014) in children with either malignant or non-malignant disorders (ClinicalTrials.gov identifier: NCT02065869).

**Material (or patients) and methods:** Included in this analysis are 21 children affected by severe combined immune deficiency (5 pts), Fanconi Anemia (5 pts), thalassemia (4 pts), Wiskott-Aldrich syndrome (3 pts) sickle cell disease (SCD), hemophagocytic lymphohistiocytosis (HLH), X-linked lymphoproliferative disorder due to XIAP mutation and Diamond-Blackfan anemia (1 pt each). Median age at haplo-HSCT was 3.9 years (range, 0.3-11.9); 11 pts (52%) were females. All pts received  $>10 \times 10^6$  CD34+ cells/Kg and  $<1 \times 10^5$   $\alpha\beta$ + T cells/Kg.

**Results:** BPX-501 cells were infused at a median time of 15 days (range 10-26) after allo-HSCT; median cell viability post-thaw was 91% (range 65-98). During the phase I portion of the study, 1 and 2 pts were given  $2.5 \times 10^5$ ,  $5 \times 10^5$  BPX-501 cells/Kg, respectively, where the remaining 18 children received  $1 \times 10^6$  BPX-501 cells/Kg (the recommended dose identified during the phase I portion to be used for the phase II portion of the study). Treatment was well tolerated and no infusion-related side effects were recorded. Two children experienced skin-only grade I acute GvHD, while no pt developed chronic GVHD. None of the 21 patients included in the analysis had secondary graft failure. Four pts with primary immunodeficiencies cleared life-threatening viral infections existing at time of starting the preparative regimen. All 21 pts are alive, after a median follow-up at time of writing of 6 months (range 1-13). All pts but 1 have full donor chimerism, the remaining child with SCD having 80% stable donor chimerism. The mean absolute number of BPX-501 cells and of CD3+ cells at 30, 60, 90, 180 and 365 days after haplo-HSCT are shown in Figure 1. In these 21 pts, the median time to reach  $0.5 \times 10^9$  CD3+ cells/L was 40 days shorter than in the hist. cts. BPX-501 cells are still persisting after their infusion in all patients.

**Conclusion:** These data indicate that the infusion of BPX-501 cells is safe and well tolerated. BPX-501 cells expand *in vivo* and persist over time, contributing to accelerate the recovery

[0007]



of adaptive immunity, with improved clinical outcome. This approach renders haplo-HSCT a safer and more attractive option for children with many different non-malignant disorders.

**Disclosure of Interest:** P. Merli: None declared, A. Bertaina: None declared, G. Li Pira: None declared, D. Pende: None declared, M. Falco: None declared, D. Pagliara: None declared, V. Bertaina: None declared, M. Sinibaldi: None declared, B. Lucarelli: None declared, L. P. Brescia: None declared, G. M. Milano: None declared, C. Cancrini: None declared, M. Montanari: None declared, S. Ceccarelli: None declared, L. Moretta: None declared, A. Moseley Employee of: Bellicum Pharmaceuticals, F. Locatelli: None declared.

#### 0008

##### Early identification of Patients with Primary Refractory Acute Myeloid Leukaemia who Benefit from Allogeneic stem cell transplantation: an analysis of 8,907 patients from the uk ncrn aml working group

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**Introduction:** Resistance to induction chemotherapy (IC) is an important adverse prognostic factor in adults with newly diagnosed acute myeloid leukaemia (AML). Despite its therapeutic relevance there is however no universally agreed definition of primary refractory AML (PREF AML). Nor has the role of allogeneic stem cell transplantation (alloSCT) been defined in this clinical setting in a large patient cohort. To address these issues we examined long term outcomes in patients with refractory AML after IC, identified using three differing diagnostic criteria.

**Material (or patients) and methods:** Response to IC was documented in 8907 newly diagnosed patients with AML treated on UK MRC and NCRN trials protocols from 1988-2012. Overall survival and predictors of outcome were studied in patients defined using the following three different possible criteria of resistance to induction chemotherapy: failure to achieve complete marrow remission (CR) after one cycle of IC (REF1A), less than a 50% reduction in blast numbers with >15% residual blasts after one cycle of IC (REF1B) and failure to achieve a CR after two courses of IC (REF2). CR was defined using conventional criteria of <5% myeloblasts 14 days following IC in a non-hypocellular bone marrow. 431 patients identified using the three criteria of refractoriness proceeded to an allogeneic SCT.

**Results:** 2548 patients failed to achieve a CR after one cycle of IC (REF1A) of whom 808 patients fulfilled REF1B criteria. 485 patients failed to achieve CR after two courses of IC (REF2).

5 year overall survival (OS) was significantly reduced in patients fulfilling any of the criteria for refractory disease when compared with patients achieving a CR following one cycle of IC. Specifically, the 5 yr OS for patients in cohorts REF1A, REF1B and REF2 was 17%, 9% and 8%, respectively, compared with 40% for patients achieving a CR after one course of IC ( $p < 0.0001$ ). Mantel-Byar analysis demonstrated that the survival of patients in the REF1B (HR 0.58 (0.46-0.74),  $P=0.00001$ ) and REF2 (HR 0.55 (0.41-0.74),  $P=0.0001$ ) cohorts was significantly improved after alloSCT compared with patients who were not transplanted.

**Conclusion:** This is the first study to assess the impact of alloSCT on outcome in a large cohort of patients with PREF AML using differing definitions of refractoriness. Our data demonstrates that alloSCT is the only treatment modality which delivers long term survival in patients with PREF AML defined using REF1B and REF2 criteria. This novel analysis of outcome in PREF AML demonstrates that patients who will benefit from alloSCT can be reliably identified by an early assessment of percentage blast reduction after the first course of IC and represents an opportunity to deliver SCT at the earliest opportunity thereby potentially improving transplant outcome.

**Disclosure of Interest:** None declared.

#### 0009

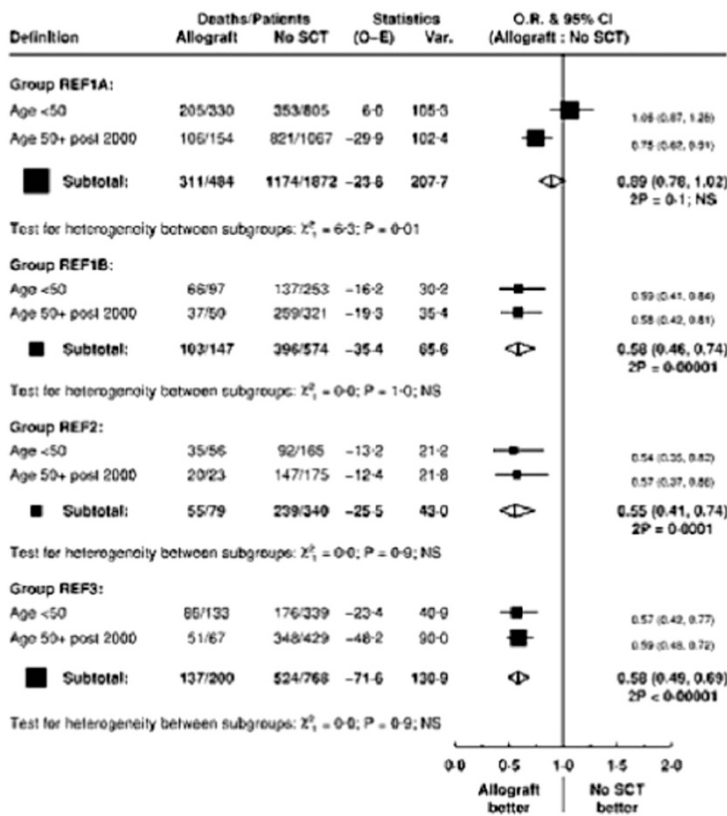
##### Definition of graft-versus-host disease-free, relapse-free survival (GRFS) for registry based studies. An ALWP- EBMT analysis on patients with acute myeloid leukemia in remission

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**Introduction:** Disease free survival is the most common used endpoint for clinical research on allogeneic stem cell transplantation (HSCT). The main pitfall of this endpoint is that it disregards event like severe graft versus host disease (GVHD), which is associated with morbidity and mortality for HSCT survivors affecting their quality of life (QoL). Therefore, a composite endpoint combining both disease-free survival and GVHD is needed. Holtan et al recently proposed to use GVHD-free, relapse-free survival (GRFS) for HSCT outcomes (1). However, chronic GVHD is a fluctuant event with time, and the start of IST may not be a good indicator of the impact of this disabling disease on long-term outcome and patients (pts) daily life. Furthermore, the major HSCT registries do not routinely collect information on start of IST.

**Material (or patients) and methods:** Therefore, we conceived a 'refined' GRFS for registry-based studies. GRFS events were defined according to the original report by Holtan, as the first event among grade III-IV acute GVHD, severe chronic GVHD, relapse and death. Then, differently from the GRFS definition by Holtan, chronic GVHD requiring systemic treatment was replaced by the occurrence of severe chronic GVHD (refined

**AML Trials: Overall Survival from Refractoriness  
Allograft versus no transplant**



GRFS). Adults transplanted for acute myeloid leukemia (AML) in first or second complete remission (CR1 or CR2), undergoing HSCT from a matched sibling donor (MSD) or matched unrelated donor (MUD) in EBMT centers from 2000 and 2014 were selected. Patients receiving myeloablative (MAC) or RIC regimen were included.

**Results:** We analyzed 20937 pts with AML the median age at HSCT was 48 years (range 18-80), and the median year of HSCT 2009 (range 2000-2014). Seventy-nine percent of the pts underwent HSCT in CR1, and 21% in CR2. Donors were MSD in 55% and MUD in 45% of pts, respectively. Stem cell source was bone marrow (BM) and peripheral blood stem cell (PBSC) in 18% and 82% of the cases, respectively. Conditioning regimen was myeloablative in 61% of the pts and reduced intensity in 39% of them, respectively. With a median follow up of 36 months (range 3-185), the probability of 3-year overall survival and DFS was 58.7% (95% CI 57.9-59.4) and 53.4% (95% CI 52.7-54.2), respectively. Cumulative incidence (CI) of relapse, non-relapse mortality, grade III-IV aGVHD and severe chronic GVHD were 27.4% (95% CI 26.7-28.1), 19.2% (95% CI 16.6-19.7), 8.3% (95% CI 7.9-8.7) and 29% (95% CI 28.4-29.7), respectively. The probability of 3-years refined GRFS (taking in account the first event occurring) was 40.1% (95% CI 39.3-40.8). Considering GRFS events, relapse accounted for the greater proportion (40%) followed by severe cGVHD accounting for 26%, NRM for 20% and grade III-IV aGVHD for 14% of the events, respectively.

**Conclusion:** Our results indicate that the modified GRFS we termed refined GRFS is a useful endpoint to evaluate HSCT outcomes. For registry based studies, the use of severe chronic GVHD is a valuable indicator of organ impairment and quality of life. For these types of studies, it could replace the use of chronic GVHD requiring systemic therapy as event for defining the GRFS. Evaluation of this new endpoint in homogenous cohort of patients and according to donor type is warranted to address risk factors that may optimize HSCT outcomes.

**References:** Holtan SG, Blood 2015.

**Disclosure of Interest:** None declared.

**O010**

**Hematopoietic Stem Cell Transplantation (HSCT) Outcomes Among Adults With Relapsed/Refractory Acute Lymphoblastic Leukemia (r/r ALL) Achieving Remission With Blinatumomab**

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**Introduction:** HSCT outcomes remain unsatisfactory in patients with ALL transplanted in the setting of r/r disease or ≥2 remission. Blinatumomab, a bispecific T-cell engager (BiTE<sup>®</sup>) antibody construct, has shown antileukemia activity in r/r ALL. Here we report outcomes from a large phase 2 study in patients with r/r ALL who received HSCT after achieving complete remission (CR) or CR with partial hematologic recovery (CRh) with blinatumomab.

**Material (or patients) and methods:** Adults with Philadelphia chromosome-negative r/r B-precursor ALL (primary refractory, relapsed < 12 months after 1<sup>st</sup> remission or HSCT, or ≥2 salvage) were eligible. Blinatumomab was given by continuous infusion (4 weeks on/2 weeks off) for up to 5 cycles. CR/CRh in the first 2 cycles was the primary

endpoint. Secondary endpoints included HSCT realization rate, 100-day post-HSCT mortality, overall survival (OS) and relapse-free survival (RFS). Individual patient data were reviewed for HSCT outcomes (additional follow-up data beyond the primary analysis were included); graft-versus-host disease (GVHD) details were not collected.

**Results:** Of 189 patients treated with blinatumomab, 83 (44%) achieved CR/CRh during the first 2 cycles. HSCT realization rate among patients in remission was 41% (34/83): 50% (27/54) in HSCT-naïve patients, 24% (7/29) in those with prior HSCT. 34 HSCT recipients (median age, 31 [18–65] years) received a median of 2 blinatumomab cycles (range 1–5) before HSCT. The majority had prior relapses (1 relapse, 62%; ≥ 2 relapses, 26%) and prior salvage therapies (1 salvage, 50%; ≥ 2 salvages, 24%). Donor types included 24 (71%) unrelated, 8 (24%) related, and 2 (6%) unknown. Details of conditioning regimens were provided for 28/34 patients: 54% myeloablative, 43% reduced intensity, and 4% unclassifiable. Conditioning regimens were initiated a median of 23 days (range 8–60) after the end of blinatumomab treatment. 27 (80%) HSCT recipients had minimal residual disease (MRD) response ( $< 10^{-4}$ ) and 26 (77%) had complete MRD response (undetectable) during the first 2 cycles. 4 (12%) patients died within 100 days post-HSCT in remission due to infection ( $n=3$ ) and GVHD ( $n=1$ ). Median OS follow-up was 13.4 months post-HSCT. 12-month KM estimates for OS and RFS were 73% (95% CI 55–85) and 53% (95% CI 34–69), respectively.

**Conclusion:** In this study, 41% of blinatumomab responders received HSCT in remission and 80% of those achieved MRD response before HSCT; RFS at 1 year was 53%. Blinatumomab can be administered in close temporal proximity to conditioning regimens without evidence of increased treatment-related mortality. In this patient group with r/r ALL, blinatumomab served as an effective salvage regimen and bridge to transplant.

**Disclosure of Interest:** A. Stein Funding from: Amgen, Conflict with: Amgen - Consultancy, Advisory Board, M. Topp Conflict with: Amgen, Roche, Affimed - Consultancy; Amgen, Jazz, Roche, Affimed - Advisory board; Amgen, Jazz - Honoraria, N. Goekbuget Funding from: Amgen, Conflict with: Amgen - Honoraria, Advisory board, R. Bargou Conflict with: Amgen - Consultancy, Honoraria, Financial Benefit and/or patents, H. Dombret Funding from: Amgen, Conflict with: Amgen - Honoraria, Consultancy, and Advisory Board, R. Larson Funding from: Amgen, A. Rambaldi Conflict with: Amgen - Honoraria, Advisory board, G. Schiller Funding from: Amgen, Conflict with: Amgen - Consultancy, Honoraria, G. Zugmaier Employee of: Amgen, L. Sterling Employee of: Amgen, J. Benjamin Employee of: Amgen, H. Kantarjian Funding from: Amgen, Novartis, Ariad, S. Forman: None declared.

## O011

### A novel second generation Chimeric Antigen Receptor (CAR) targeting CD19 antigen to redirected T cells toward pre-B neoplasia

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**Introduction:** Genetic engineering of T cells to express CARs is a promising new approach for adoptive immunotherapy of cancer. Clinical trials have demonstrated impressive activity of CD19-CAR T cells against B-cell malignancies. In particular, CAR CD19 T-cells could represent a new therapeutic strategy that combines a greater efficacy and lower toxicity than conventional therapy. Commonly, second generation CARs employed in the current US clinical trials, incorporate one costimulatory domain, either CD28 or 4.1bb with the CD3- $\zeta$  sequence in a lentiviral vector, for full activation of T cells. In our study, we evaluate the anti-leukemia effect of a second generation CAR. CD19-4.1bb. $\zeta$  retroviral vector in combination with the suicide

gene inducible caspase 9 (iC9), as a safety switch for adverse events.

**Material (or patients) and methods:** Single chain variable Fragment specific for CD19 was cloned in frame with CD8 transmembrane domain, 4.1bb costimulatory domain and CD3- $\zeta$  sequence (CAR.CD19-4.1bb. $\zeta$  retroviral vector). Moreover, we add the CD34 as selectable marker and the new suicide gene iC9. Then, activated T cells were retrovirally transduced and analyzed by FACS, using an anti-CD34 antibody. Cr<sup>51</sup> assays and *in vitro* co-cultures were carried out to evaluate CAR-T cell anti-leukemia effect. iC9 activation was evaluated by AnnexinV/7AAD staining after exposure to AP1903, the dimerizing activating iC9. To assess the expansion, persistence, and antitumor effect of transgenic CAR.CD19-4.1bb. $\zeta$  T cells *in vivo*, we used a SCID mouse model engrafted with luciferase-positive CD19+ human leukemia cell lines.

**Results:** Polyclonal T cells genetically modified by CAR.CD19-4.1bb. $\zeta$  show a cytokine-dependent expansion at the same level of control un-transduced T cells (NT). Flow-cytometric analysis of T cells stained with anti-CD34 identified from 62% to 91% CAR.CD19 transduced T cells (mean, 85%). Whereas NT cells were incapable of lysing CD19+ tumor targets (0% > 5%), 49%, 58% and 68% of BV173, Raji and Daudi cells (all CD19+ LLA derived cell lines) were lysed during co-incubation with CAR.CD19-4.1bb. $\zeta$  T cells at the E/T ratio of 20:1. No cytolytic activity of transduced cells against CD19- Karpas or K562 cell lines was seen. Moreover, co-culture experiments performed for a period of 7 to 15 days, show that CAR.CD19-4.1bb. $\zeta$  T cells were capable to significantly control overtime the growth of CD19+ targets, since only 0.1% and 3.2% of CD19+ Daudi and Raji cells, respectively, survive after incubation with CAR.CD19-4.1bb. $\zeta$  T cells. Indeed, 85% and 82% of Daudi and Raji cells, were still present when incubated with NT cells. Addition of AP1903 eliminates CAR.CD19-4.1bb. $\zeta$  T cell activity and induce apoptosis in 96% of the trasduced cells. NGS mice infused with Raji cell line and treated with CAR.CD19-4.1bb. $\zeta$  T cells, show a bioluminescence of  $3.8 \times 10^5 \pm 3.8 \times 10^4$  by day 17, significantly reduced in comparison with mice treated with NT ( $3.2 \times 10^8 \pm 2.8 \times 10^7$  by day 17).

**Conclusion:** T cells redirected with a CAR that specifically targets the CD19 antigen, and incorporates 4.1bb costimulation demonstrated survival, expansion and anti-tumor activity *in vitro* and *in vivo* in the presence of CD19+ leukemia cells. Importantly, the incorporation of an inducible suicide gene and its pharmacologic activation efficiently eliminated these gene-modified T cells, this increasing the safety of the proposed approach.

**Disclosure of Interest:** I. Caruana: None declared, B. De Angelis: None declared, D. Orlando: None declared, I. Boffa: None declared, M. Guercio: None declared, V. Polito: None declared, A. Moseley Employee of: Bellicum Pharmaceuticals, Inc, F. Locatelli: None declared, C. Quintarelli: None declared.

## O012

### Comparison of Matched/Mismatched Unrelated Donor Stem Cell Transplantation to Autologous Stem Cell Transplantation for Acute Myeloid Leukemia in First Complete Remission: A Study from the Acute Leukemia Working Party of the EBMT

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**Introduction:** As AML post-remission strategy, allogeneic stem cell transplant is the most effective treatment to prevent leukemia relapse, and for patients lacking a matched sibling donor, transplantation from an unrelated donor (URD) is usually the preferred alternative. Autologous Stem Cell Transplant (ASCT) is an alternative option, with several advantages including low non relapse mortality (NRM), no GVHD risk and better quality of life.

**Material (or patients) and methods:** We performed a retrospective analysis of 2689 AML patients receiving 10/10 URD ( $n=1260$ ), 9/10 URD-HSCT ( $n=356$ ) or ASCT ( $n=1073$ ) in first CR between 2005 and 2013 and reported to the EBMT.

**Results:** Median FU was 35 months for ASCT, 27 months for 10/10 and 9/10 URD. The 2-year relapse incidence (RI) for ASCT, 10/10 and 9/10 URD were  $46 \pm 3\%$ ,  $25 \pm 3\%$  and  $28 \pm 5\%$ , respectively ( $P < 10^{-5}$ ), while the 2-year NRM rates were  $3 \pm 2\%$ ,  $16 \pm 4\%$  and  $21 \pm 4\%$ , respectively ( $P < 10^{-5}$ ). The 2-year LFS was  $51 \pm 3\%$  for ASCT,  $59 \pm 3\%$  for 10/10 URD and  $52 \pm 6\%$  for 9/10 URD ( $P=0.002$ ), while the 2-year OS was  $68 \pm 3$ ,  $63 \pm 3\%$  and  $55 \pm 6\%$ , respectively ( $P < 10^{-4}$ ). 10/10 URD showed significantly lower RI and better LFS compared to ASCT independently of cytogenetic risk. In favourable risk group, ASCT provided good outcome, with 2y LFS and OS of  $61 \pm 6\%$  and  $80 \pm 6\%$ , respectively. In intermediate risk subgroup, ASCT resulted in similar OS compared to 10/10 URD and better OS compared to 9/10 URD ( $66 \pm 4\%$  for ASCT,  $66 \pm 5\%$  for 10/10 URD,  $55 \pm 7\%$  for 9/10 URD,  $P=0.012$ ) (Fig 1). FLT3 mutational status affected outcome; in patients with wild type FLT3-ITD URD showed better LFS compared to ASCT ( $51 \pm 8\%$ ,  $67 \pm 7\%$ ,  $64 \pm 13\%$  for ASCT, 10/10 and 9/10 URD, respectively,  $P=0.008$ ) but no difference was observed in OS ( $72 \pm 7\%$ ,  $72 \pm 6\%$ ,  $65 \pm 13\%$ , respectively,  $P=0.87$ ), while in patients harboring FLT3-ITD 10/10 URD showed better LFS and OS compared to ASCT. Interestingly, when comparing outcome of the subgroup of patients who received reduced intensity conditioning-URD (RIC-URD) with ASCT, we observed no difference in LFS ( $51 \pm 4\%$ ,  $58 \pm 4\%$ ,  $48 \pm 8\%$  for ASCT, 10/10 and 9/10 URD, respectively,  $P=0.07$ ). Multivariate analysis confirmed significantly lower RI for 10/10 (HR 0.36,  $P < 10^{-5}$ ) and 9/10 URD (HR 0.43,  $P < 10^{-5}$ ) and higher NRM for 10/10 URD (HR 3.88,  $P < 10^{-5}$ ) and 9/10 URD (HR 4.89,  $P < 10^{-5}$ ) compared to ASCT. URD-SCT was associated with better LFS compared to ASCT (HR 0.57,  $P < 10^{-5}$ , for 10/10 URD; HR 0.69,  $P=0.002$  for 9/10 URD). 10/10 URD was associated with better OS compared to ASCT (HR 0.81,  $P=0.031$ ) but no difference in OS was observed between 9/10 URD and ASCT (HR 1.02,  $P=0.87$ ).

**Conclusion:** In AML patients lacking an HLA-matched sibling donor URD-HSCT significantly reduces relapse risk and improves LFS. 10/10 URD showed better OS compared to ASCT in MV analysis in our series, while 9/10 URD impact on

LFS didn't translate in better OS. In intermediate risk patients, in the absence of an HLA fully matched sibling or unrelated donor, autologous transplant may be considered as a valid option as ASCT results seem to overlap 10/10 URD outcome and to provide better survival compared to mismatch URD.

**Disclosure of Interest:** None declared.

### O013

#### Improved survival after allogeneic stem cell transplantation compared to chemotherapy alone in patients with acute myeloid leukemia patients in first remission. A nationwide population-based study

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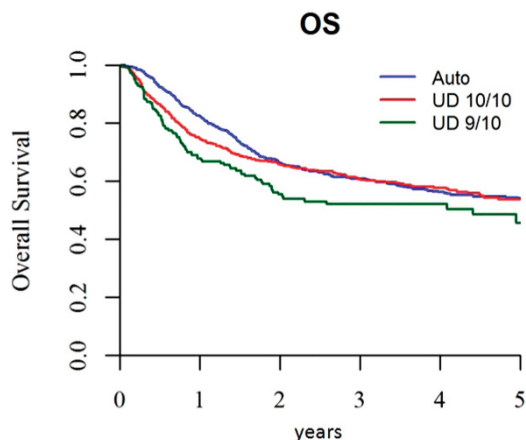
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**Introduction:** There is a lack of randomized studies of allogeneic HSCT versus chemotherapy alone for AML in CR1. One approach is the "donor-no donor" method, but results are conflicting, especially in relation to cytogenetic risk groups. We utilized a nation-wide Danish registry for a "transplant-no transplant" analysis.

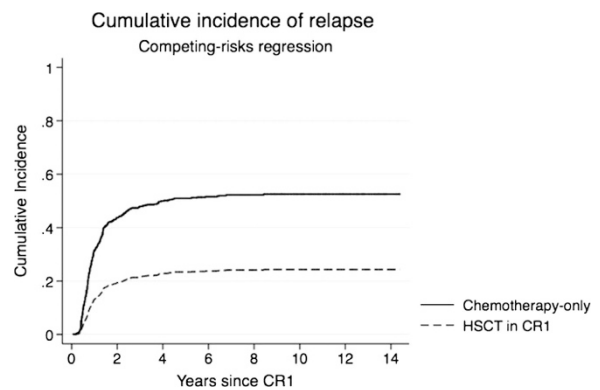
**Material (or patients) and methods:** Using the Danish National Acute Leukemia Registry ( $n=3205$ ), we compared relapse risk, relapse-free survival (RFS), non-relapse mortality, and overall survival between patients receiving conventional chemotherapy-only and patients undergoing HSCT in CR1. To avoid immortal time bias and to mitigate confounding, we used both a landmark approach and propensity weighted and matched analyses.

Results were adjusted for age, gender, WBC at diagnosis, Karnofsky performance score, AML/sAML/tAML, comorbidity and length of hospital stay. Results are presented overall and stratified by age ( $< 60$ ,  $\geq 60$  years) and cytogenetic risk group, with corresponding 95% confidence intervals (CI).

**Results:** In total, 830 patients achieved a CR1 and were alive and free of relapse at the median time from diagnosis to HSCT (183 days). Of these, 176 patients (21.2%) underwent HSCT. HSCT significantly improved relapse-rates (22.3% versus 50.5%, figure), whereas no difference was found in median-time to relapse (308 days versus 280 days). Relapse-free survival and overall survival were superior among patients receiving HSCT in all subgroups (overall survival, adjusted mortality ratios (MRs):  $< 60$  years, 0.65 (CI=0.46-0.90),  $\geq 60$  years, 0.56 (CI=0.35-0.89), intermediate risk cytogenetics, 0.62 (CI=0.44-0.88), and adverse risk cytogenetics, 0.55 (CI=0.33-0.92)).



**Figure 1.** Overall survival in patients with intermediate risk cytogenetics.





The improved overall survival was achieved by a substantial reduction in relapse risk, as shown in the table.

	Relapse risk crude	Relapse risk adjusted
<i>All patients</i>		
HSCT in CR1	0.37 (0.27-0.52)	0.34 (0.24-0.49)
Chemotherapy-only	1	1
<i>Intermediate risk</i>		
HSCT in CR1	0.26 (0.16-0.43)	0.26 (0.16-0.43)
Chemotherapy-only	1	1
<i>Adverse risk</i>		
HSCT in CR1	0.56 (0.32-0.99)	0.55 (0.31-0.98)
Chemotherapy-only	1	1

**Conclusion:** In conclusion, our findings suggest that the anti-leukemic effect of HSCT in a population-based nation-wide cohort improves outcomes in a large fraction of both younger and older patients with intermediate and adverse cytogenetic risk group features.

**Disclosure of Interest:** None declared.

#### O014

##### The hierarchy of alternative donors for allogeneic hematopoietic stem cell transplantation in poor risk AML in CR1 revisited

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**Introduction:** Allogeneic hematopoietic stem cell transplantation (alloHSCT) with either matched related donors (MRD) or alternative donors is the most effective post-remission treatment (PRT) for prevention of relapse in poor risk acute myeloid leukemia (AML) in first complete remission (CR1). We set out to compare outcome in patients with poor risk AML in CR1 receiving alloHSCT using either MRD, 10/10 or 9/10 matched unrelated donors (MUD), haplo-identical alloHSCT or umbilical cord blood transplantation (UCBT) with either low or high ( $\leq$  vs  $>$   $3.0 \times 10^7$ /kg) total nuclear cell count (TNC) at infusion in order to establish the hierarchy of alternative donors.

**Material (or patients) and methods:** 3377 adult patients, transplanted between 2000 and 2013 and reported to the EBMT Acute Leukemia Working Party, with poor risk AML in CR1 receiving an alloHSCT using MRD ( $n=2061$ ) or alternative donors, including 10/10 ( $n=827$ ) or 9/10 MUDs ( $n=248$ ), UCBT with low TNC ( $n=61$ ) or high TNC ( $n=89$ ), or haplo ( $n=69$ ) were eligible for the analysis. Poor risk AML was defined as either white blood cell count  $>100 \times 10^9$ /L at diagnosis, secondary AML, cytogenetic abnormalities associated with adverse risk, according to European LeukemiaNET classification or no CR after one cycle of induction chemotherapy.

**Results:** Recipients of haplo alloHSCT and UCBT were younger than MRD alloHSCT recipients (median age, 45 and 44 versus 48 years, respectively,  $P=0.002$ ). Both reduced intensity conditioning and myeloablative conditioning (MAC) were applied, with a higher percentage of MAC in recipients of haplo (59%) and MRD (62%) alloHSCT versus patients receiving MUD alloHSCT (51%) or UCBT with low (52%) or high TNC (38%). 4 year OS following MRD alloHSCT was 53%, which did not differ from 10/10 MUD (52%) and haplo alloHSCT (55%). OS, however, was significantly lower for 9/10 MUD alloHSCT (45%) and UCBT with low (41%), and high TNC (42%), respectively ( $P=0.010$ ). 4 year RFS was 48%, 47% and 51% following MRD, 10/10 MUD, and haplo alloHSCT, respectively, which was significantly ( $P=0.023$ ) better than following UCBT with low (31%), and high TNC (37%), or 9/10 MUD alloHSCT (43%). 4 year NRM depended on donor type and estimated 26%, 29% and 25% after haplo alloHSCT, UCBT with low and high TNC, respectively, versus 16% following MRD alloHSCT. Multivariable analysis confirmed the impact of donor type with OS following MRD, 10/10 MUD, and haplo alloHSCT being not statistically significantly different. Relapse did not significantly differ by donor type in multivariable analysis, while NRM was significantly higher for alternative donors as compared with MRD alloHSCT.

**Conclusion:** Collectively, these results suggest that alloHSCT with MRDs and 10/10 MUDs may still be preferred in patients with poor risk AML in CR1. If an HLA matched related or unrelated donor is not available, then the repertoire of alternative donors includes 9/10 MUD, UCBT, and haplo-identical donors. The latter type of donor is increasingly applied and now approximates results with matched donors. However, longer follow-up after haplo-identical donor transplantation may be needed to definitely establish its new place in the hierarchy of alternative donors.

**Disclosure of Interest:** None declared.

#### O015

##### Anti-CD19 BiTE Blinatumomab Treatment in Adults with Relapsed/Refractory B Precursor Acute Lymphoblastic Leukemia (r/r ALL) Post-Allogeneic Hematopoietic Stem Cell Transplantation

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**Introduction:** Current therapies for patients with r/r ALL who have had prior allogeneic hematopoietic stem cell transplantation (alloHSCT) have very poor outcomes. Blinatumomab, a bispecific T-cell engager (BiTE) antibody construct, has shown antileukemia activity in r/r ALL. Here we characterize patients with r/r ALL and prior alloHSCT before blinatumomab treatment from a large phase 2 study.

**Material (or patients) and methods:** Eligible adults had Philadelphia chromosome-negative r/r ALL and one of the following negative prognostic factors: primary refractory, relapsed  $<$  12 months after 1<sup>st</sup> remission or alloHSCT, or  $\geq$  2

salvage. All patients stopped immunosuppressive graft-versus-host disease (GvHD) therapy within 2 weeks of treatment start; those with active GvHD were excluded. 189 patients received blinatumomab by continuous IV infusion (4 weeks on/2 weeks off) for up to 5 cycles. Complete remission (CR) or CR with partial hematologic recovery (CRh) within the first 2 cycles was the primary endpoint. Secondary endpoints included overall survival (OS), relapse-free survival (RFS) and adverse events (AEs).

**Results:** A total of 64 (34%) of 189 patients had received alloHSCT before enrollment; 10 had 2 prior alloHSCTs. Donor types primarily included 29 (45%) matched sibling and 31 (48%) unrelated; 59% received myeloablative conditioning regimens. Median age was 32 (range 19–74) years. At baseline, 23 (36%) patients had 1 prior relapse, 24 (38%) had 2 prior relapses, and 17 (27%) had  $\geq 3$  prior relapses; 28 (44%) patients had relapsed post-alloHSCT. Of 55 patients with previous salvage, 38 (69%) had received salvage therapy after last alloHSCT and before blinatumomab. Median time between the last alloHSCT or salvage to relapse was 6 (1–33) months. Median time from last prior alloHSCT to first dose of blinatumomab was 10 (3–40) months. 19 (30%) patients had a history of GvHD, and 42 (66%) had  $\geq 50\%$  bone marrow blasts (central laboratory). Patients received blinatumomab for a median of 2 (1–5) cycles. Efficacy data are presented below.

Summary of efficacy (N = 64)

Patients achieving CR/CRh within first 2 cycles of blinatumomab treatment, n (%)	29 (45)
CR	18 (28)
CRh	11 (17)
MRD response <sup>a</sup> , n (%)	22 (76)
MRD complete response <sup>a</sup>	19 (66)
Median RFS <sup>a</sup> , months (95% CI)	6.1 (5.0–7.7)
Median OS, months (95% CI)	8.4 (4.2–9.4)

<sup>a</sup>in patients achieving CR/CRh.

In total, 56 (88%) had grade  $\geq 3$  treatment-emergent (TE)AEs, including neutropenia (22%), febrile neutropenia (20%), anemia (17%) and thrombocytopenia (14%). Six patients reported GvHD (2 grade  $\geq 3$ ) during blinatumomab treatment (3 GvHD in skin). Eight patients had fatal TEAEs (gastrointestinal hemorrhage, respiratory failure, 6 infection/infestation); one (candida infection) was considered possibly related to treatment by the investigator. None of the fatal TEAEs occurred during remission.

**Conclusion:** In this heavily pretreated patient group with r/r ALL and prior alloHSCT, single-agent blinatumomab induced a 45% CR/CRh rate, with an AE profile consistent with that previously reported.

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Consultancy, Honoraria and Patents & Royalties: Patent for blinatumomab, Pfizer - Consultancy and Honoraria, Novartis - Consultancy and Honoraria, GEMoB GmbH - Consultancy and Honoraria, M. Litzow: None declared, A. Rambaldi Funding from: Celgene, Conflict with: Amgen - Honoraria, Novartis - Honoraria, Roche - Honoraria, Pierre Fabre - Honoraria, J.-M. Ribera Funding from: Amgen, Pfizer, A. Zhang Employee of: Amgen, Conflict with: Amgen - Equity Ownership, Z. Zimmerman Employee of: Amgen, Conflict with: Amgen - Equity Ownership, S. Forman Funding from: Mustang, Conflict with: Amgen - Consultancy.

**O016**

**Allogeneic Stem Cell Transplantation Offers Similar Outcome After Myeloablative and Sequential Conditioning Regimen in Patients with Refractory or Relapsed Acute Myeloid Leukemia (AML): a study from the SFGM-TC**

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**Introduction:** AML patients failing to achieve complete remission (CR) or relapsing after intensive chemotherapy (CHEMO), have a dismal prognosis. In this setting, allogeneic hematopoietic cell transplantation (HCT) is a curative salvage therapy, but myeloablative conditioning (MAC) transplants are associated with high non-relapse mortality (NRM). For young patients, there is no evidence to prefer MAC HCT to a sequential (SEQ) HCT consisting of cytoreductive CHEMO, followed by reduced-intensity conditioning (RIC) and prophylactic transfusion of donor lymphocytes (DLI). The objective, analysing the data from SFGM-TC, was to compare the results of these 2 approaches in patients transplanted in AML not in CR.

**Material (or patients) and methods:** Inclusion criteria: a) HCT performed from January 2006 to December 2013 b) Patient age: 18-50 years (y), c) Transplant for refractory or relapsed AML, d) SEQ or MAC regimen e) Matched sibling donor, matched or mismatched unrelated donor.

Patients characteristics: 99 patients (median age: 40 y (range 18-50)) were analysed. At diagnosis, 4% of patients were classified favorable, 34% intermediate-1, 24% intermediate-2, and 38% adverse, according to the European LeukemiaNet (ELN) score. Before HCT, patients received a median of 2 lines of CHEMO (range 0-3). At transplant, 51.5% of patients were in induction failure and 48.5% in relapse. The median of bone marrow (BM) and circulating blast percentages were 20% (range 0-96) and 2% (range 0-93) respectively.

Transplant modalities: 58 patients received a SEQ approach (CHEMO with FLAMSA ( $N=39$ ) or with clofarabine ( $N=19$ ) + high-dose cytarabine, followed by RIC combining cyclophosphamide (CY) with 4 Gy total body irradiation (TBI) or with busulfan (BU)) while 41 patients received a MAC regimen (CY combined with 10-12 Gy TBI ( $N=17$ ) or with BU ( $N=24$ )). Forty-one percent of patients were transplanted from a sibling and 59% from an unrelated donor. Stem cell source was peripheral blood in 79% and BM in 21%.

**Results:** MAC and SEQ groups had similar ages, ELN scores, numbers of pre-transplant CHEMO lines and status at HCT. They only differed from their circulating blasts at HCT (median 1%, range (0-85) in MAC group vs 8%, range (0-93) in SEQ group,  $P=0.02$ ). In univariate analysis, type of conditioning regimen (MAC vs SEQ) did not impact post-transplant outcomes: 2-y overall survival was 38.7% (95% confidence interval (CI): 24-53.2%) in MAC patients vs 32.5% (95%CI: 20.9-44.6%) in SEQ patients ( $P=0.39$ ); 2-y cumulative incidence of relapse was 56.7% (95%CI: 41-72.4%) vs 50% (95%CI: 36.9-63.1%) respectively ( $P=0.99$ ) and 2-y non-relapse mortality was 14.8% (95%CI: 3.6-26%) vs 17.2% (95%CI: 7.3-27.1%) respectively ( $P=0.44$ ). Grade II-IV acute graft versus host disease (GvHD) occurred more frequently in patients who received MAC: 68% vs 36% in the SEQ group ( $P=0.002$ ), as did chronic-GvHD (44% vs 24%,  $P=0.038$ ). Multivariate analysis identified relapse status (hazard ratio (HR) 1.9, 95%CI 1.1-3.1,  $P=0.022$ ) and high percentage of circulating blasts at transplant (HR 1.01, 95%CI 1.003-1.02,  $P=0.008$ ), as factors independently associated with poor OS. Only 6 patients received DLI (5 in SEQ and 1 in MAC group) and 5 relapsed.

**Conclusion:** In conclusion, both MAC and SEQ regimens offer similar outcomes, without increased NRM after MAC transplant.

**Disclosure of Interest:** None declared.

#### O017

##### Sequential intensified conditioning regimen allogeneic hematopoietic stem cell transplantation in adult patients with high-risk AML in complete remission: a survey from the ALWP of the EBMT

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**Introduction:** Post-transplant relapse remains a challenge after RIC, particularly in AML patients with adverse prognosis factors. The so-called "sequential" transplant approach (e.g. FLAMSA regimen combining both intensive chemotherapy and reduced intensity conditioning (RIC) HCT within the same procedure) initially developed in patients with refractory AML, could be a promising strategy to improve disease control and decrease the risk of relapse in high-risk AML patients in complete remission (CR) not eligible to standard myeloablative conditioning.

**Material (or patients) and methods:** In the current study we analyzed transplantation outcomes in a cohort of 265 adults patients transplanted between 2002 and 2014 for high-risk AML in CR (intermediate or high risk cytogenetic or secondary AML). Patients received a "sequential" conditioning regimen based on Fludarabine 30 mg/m<sup>2</sup>/d, high-dose aracytine 1-2 g/m<sup>2</sup>/d, amsacrine 100 mg/m<sup>2</sup>/d for 5 days and after a 3 days rest, TBI 4Gy, cyclophosphamide 50-120 mg/kg, and ATG for 2 to 3 days (TBI group,  $n=159$  [60%]). In 106 (40%)

patients, TBI was substituted by IV Busulfan 3.2 mg/kg/d for 2 days, or orally equivalent dose (Bu group). At time of transplant, 216 (81%) patients were in CR1 and 49 (19%) in CR2. Cytogenetic in de novo AML was intermediate in 114 patients (43%), and poor in 42 (16%), 109 patients (42%) had secondary AML. 74 (28%) patients received MRD and 191 (72%) unrelated donor URD HCT. Majority of patients (95%) received mobilized PBSC.

**Results:** Median follow-up of surviving patients was 46 months and median age at transplant was 55 years (19-76). Eight patients (3%) failed to engraft. Two year cumulative incidence of relapse (RI) was 21% (95%CI, 15-28%) in the TBI group versus 26% (95%CI, 17-35%) in the Bu group. ( $P=0.77$ ). NRM was significantly lower in the TBI group 19% (95%CI, 13-26%) versus 31% (95%CI, 24-38%) in the Bu group ( $P=0.02$ ). LFS and OS at 2 years were 59% (95%CI, 51-67%) versus 43% (95%CI, 33-54%;  $P=0.14$ ) and 62% (95%CI, 54-70%) versus 47% (95%CI, 36-57%;  $P=0.14$ ) in the TBI and Bu groups respectively. Acute GVHD (grade II-IV) incidence was 30% (95%CI, 23-38%) in the TBI group versus 26% (95%CI, 18-35%) in the Bu group ( $P=0.45$ ). The 2-year cumulative incidence of chronic GVHD was 34% (95%CI, 26-41) in the TBI group versus 29% (95%CI, 20-39) in the Bu group ( $P=0.79$ ). In multivariate analysis adjusted for variable with different distribution between Bu and TBI groups, the type of conditioning (TBI vs Bu) has no impact on RI, NRM, LFS and OS. Older age at transplant was an independent adverse prognostic factor in multivariate analysis for OS (hazard ratio (HR): 1.21, 95% CI: 1.01-1.45,  $P=0.04$ ). Having a poor cytogenetic status was associated with a significant lower OS (HR: 1.76, 95% CI: 1.06-2.92,  $P=0.03$ ) and higher RI (HR: 1.96, 95% CI: 1.03-3.72,  $P=0.04$ ). Of note, CR1 vs. CR2 had no impact on patients' outcome.

**Conclusion:** These results in a rather large cohort of patients with AML suggest that a FLAMSA "sequential" regimen provided an efficient disease control in high-risk AML patients including in CR2 and secondary AML. Furthermore Busulfan and TBI based FLAMSA "sequential" regimens provide a similar outcome. These results should be confirmed in a multicenter well design randomized study.

**Disclosure of Interest:** None declared.

#### O018

##### Thiotepa-based conditioning for allogeneic stem cell transplantation (allo-HSCT) in acute lymphoblastic leukaemia (ALL) - a survey and matched-pair analysis from the ALWP of the EBMT

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**Introduction:** Thiotepa is an alkylating compound with an antineoplastic activity that was used in the past mainly for solid tumors and lymphoma, partially due to its ability to penetrate the blood-brain-barrier. Moreover, beside its myelosuppressive activities, thiotepa has immunosuppressive

properties making it an attractive agent to be used in the conditioning pre-transplantation. In this retrospective study, we analysed thiotepa-based conditioning regimen for allo-HSCT in adult patients with ALL using the EBMT database.

**Material (or patients) and methods:** Inclusion criteria were: adults with de novo or secondary ALL who underwent first allo-HSCT between 2000 and July 2014 with a thiotepa-based regimen. Donors were either HLA-matched siblings or matched unrelated donors.

**Results:** A total of 323 patients were identified. Median age was 43 years (range, 18 – 76) and 59% were males. Disease status at allo-HSCT was CR1 in 48.9%, CR2 in 21.7%, CR3 in 6.2% and 23.2% of the patients had an active disease at time of transplant. Transplantation was performed from a HLA-matched sibling (49.8%) or a matched unrelated donor (51.2%). Sixty-five per cent of patients received a myeloablative and 35% a reduced-intensity conditioning regimen, respectively. Stem cell source was peripheral blood stem cells in 84% of the transplants, while 16% received bone marrow grafts.

Incidence of acute graft-versus-host disease (GvHD) grade > II was 26.6%, while chronic GvHD occurred in 35.9% at one year (24.6% with extensive disease). With a median follow-up of 16.8 months, the non-relapse mortality (NRM) at one year was 25.3%. Relapse incidence (RI) at one year was 33.3% and the one-year leukemia-free survival (LFS) and overall survival (OS) incidences were 57% and 66%, respectively.

We then performed a matched-pair analysis, comparing myeloablative thiotepa-based regimens with total body irradiation/cyclophosphamide (TBI/Cy) and could identify 226 patients in the thiotepa group, matched with 678 patients in the TBI/Cy group. Acute GvHD grade > II was observed in 11.2% in the thiotepa group versus 17.4% after TBI/Cy ( $P=0.071$ ). One-year cumulative incidence of chronic GvHD was 38.5% for thiotepa and 41.5% for TBI/Cy, respectively ( $P=0.106$ ). At one year, the cumulative incidences of NRM and RI were 22.8% with thiotepa versus 19.7% with TBI/Cy ( $P=0.935$ ) and 33.2% (thiotepa) versus 28.6% (TBI/Cy;  $P=0.0573$ ), retrospectively. Also, the probabilities of LFS and OS at one year were not significantly different between the thiotepa and TBI/Cy group, at 44% versus 51.6% ( $P=0.24$ ) and 57.2% versus 62.6% ( $P=0.91$ ), respectively.

Table 1 shows the outcome at one year according to disease status

		NRM		RI		LFS		OS
<i>in CR1</i>								
Thiotepa	22.8%	$P=0.913$	28.6%	$P=0.0191$	48.6%	$P=0.19$	65.5%	$P=0.84$
TBI/Cy	17.1%		18.8%		64.1%		75.5%	
<i>in CR2+</i>								
Thiotepa	22.7%	$P=0.797$	38.9%	$P=0.587$	38.4%	$P=0.85$	47.2	$P=0.44$
TBI/Cy	22.8%		40.5%		36.6%		46.9%	

**Conclusion:** This large survey suggests that thiotepa-based conditioning therapy in adult ALL is feasible and effective. Furthermore, a matched-pair analysis between thiotepa-based and TBI/Cy-based myeloablative conditioning therapy showed similar main outcomes in both groups. In the subgroup of patients transplanted in CR1, RI was higher in the thiotepa group, whereas no difference was observed in patients in CR2+. The value of thiotepa-based conditioning should be verified in a prospective trial.

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## O019

### Romidepsin (Rom) Enhances the Cytotoxicity of Fludarabine (Flu), Clofarabine (Clo) and Busulfan (Bu) in Malignant T-cells

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**Introduction:** T-cell malignancies are a rare group of lymphoid hematologic disorders. Patients with relapsed disease or aggressive subtypes have poor outcomes, even with stem cell transplantation. In an effort to identify a more efficacious conditioning regimen, we investigated the synergistic cytotoxicity of the nucleoside analogs Flu and Clo, the DNA alkylating agent Bu, and the histone deacetylase inhibitor Rom in malignant established T-cell lines and patient samples.

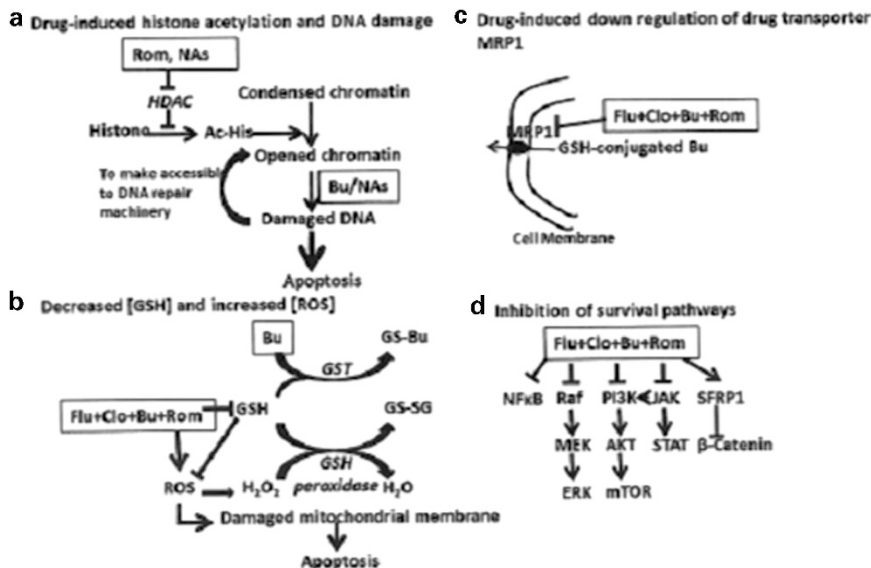
**Material (or patients) and methods:** PEER and SUPT1 T-ALL cell lines were exposed to drugs individually (IC<sub>10-20</sub>) or in combination for 48 hrs and analyzed by the MTT proliferation assay, flow cytometry, and Western blotting. Mononuclear blast cells were isolated from peripheral blood samples of patients with refractory T-cell malignancies.

**Results:** PEER cells exposed to individual drugs, [Flu+Bu], [Clo+Bu], or [Flu+Clo+Bu] had  $\geq 75\%$  survival. A lower survival (~30%) was observed with [Flu+Clo+Bu+Rom], which correlates with increased Annexin V positivity (~65%). SUPT1 cells had ~12% survival and ~46% Ann V-positivity in [Flu+Clo+Bu+Rom] whereas the individual drugs and other combinations showed less cytotoxicity. Combination indexes of 0.4 – 0.5 at 50% drug effect were obtained for [Flu+Clo+Bu+Rom] in both PEER and SUPT1, suggesting strong synergism. Exposure of cells to the 4-drug combination resulted in a significant induction of histone modifications, activation of DNA-damage response (DDR), production of reactive oxygen species (ROS), decreased glutathione (GSH) levels and mitochondrial membrane (MM) potential, and activation of apoptosis. A similar activation of DDR and apoptosis was observed in patient samples. In cell lines, the NF $\kappa$ B, Raf-MEK-ERK, PI3K-AKT-mTOR, JAK-STAT, and Wnt/ $\beta$ -catenin prosurvival signaling pathways were inhibited. A novel finding that may also explain the observed synergism was the inhibition of expression of the drug transporter MRP1 by Flu and Rom. MRP1 is known to mediate the efflux of GSH-conjugated Bu. [Flu+Clo+Bu+Rom] dramatically decreased the level of MRP1 suggesting a possible increase in the intracellular concentration of free, unconjugated Bu.

**Conclusion:** Based on above, we propose four mechanisms of synergism (Figure). (A) Flu, Clo and Rom induce histone modifications and concomitant chromatin remodeling, which increases access of Bu and Flu-triphosphate to DNA. (B) The increased production of ROS, likely due to drug-mediated stress response and decreased GSH, damages the MM causing leakage of pro-apoptotic factors. (C) Down-regulation of MRP1 results in increased intracellular concentration of free Bu, exacerbating DDR. (D) Inhibition of pro-survival pathways. These mechanisms converge to activation of caspases and apoptosis. In summary, our results may be used as basis for a clinical trial using these drugs as conditioning therapy for patients with advanced T-cell malignancies.

**Disclosure of Interest:** None declared.

[0019]



**0020**  
**Long-Term Survival and Late Events after Allogeneic Stem-Cell Transplantation (SCT) for AML with Myeloablative (MAC) Compared to Reduced-Intensity Conditioning (RIC). A report on behalf of the ALWP of EBMT**

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**Introduction:** Allogeneic SCT is curative therapy in AML. Most deaths after SCT occur within the first 2 years. Prior large cohort studies showed that patients (pts) surviving leukemia free 2 years after MAC have high probability of survival at 10 years. RIC emerged as effective modality in AML. Several studies showed similar leukemia-free survival (LFS) following MAC and RIC. However, there is paucity of data on the long-term outcome (beyond 10 years) and the pattern of late events following RIC due to the relative recent introduction of this approach.

**Material (or patients) and methods:**

We analyzed long-term outcomes in a relatively large cohort of pts with AML(n=1423), age ≥ 50 years, after SCT from matched siblings, in the years 1997-2005, median follow up 8.3 years (0.1-17).

**Results:** The 10-yr LFS was 31% (95CI, 27-35) and 32% (28-35) after MAC and RIC, respectively (P=0.57). The 10-yr GVHD/relapse free survival (GRFS), a surrogate for quality of life was 22%(18-25) and 21%(18-24), respectively (P=0.79). 504 pts were alive and leukemia-free 2 years after SCT, 287 after MAC and 297 after RIC. The 10-yr LFS of pts surviving leukemia-free at the 2 year landmark was 71%(65-76) and 73%(67-78) after MAC and RIC, respectively (P=0.76). Multivariate analysis (MVA) identified advanced disease at SCT (HR 1.9, P=0.01) and female donor to male recipient (HR 1.5, P=0.04) as independent factors predicting LFS. The conditioning regimen, age, cytogenetics and prior chronic GVHD were not significant. The 10-yr overall survival (OS) was 73%(67-78) and 74%(69-80), respectively (P=0.81). Advanced disease was the only predicting factor in MVA (HR 2.0, P=0.01). There were 86 late deaths after MAC, 53 in years 3-5 and 33 beyond 5 years. 97 deaths occurred after RIC, 67 in years 3-5 and 30 beyond 5 years. Relapse was the leading cause of late death after both regimens. It was the cause of 72% and 87% of deaths in years 3-5 after MAC and RIC, respectively (P=0.06), and 42% and 83% of deaths beyond 5 years, respectively (P=0.006). In all the 10-yr relapse rate was 14%(10-19) and 19%(14-24), respectively (P=0.12). MVA identified disease status at SCT (P=0.02) and poor cytogenetics (P=0.04) as factors predicting for relapse. The regimen used was not predictive. Prior chronic GVHD was no longer protective against relapse in pts reaching the 2-year landmark leukemia free. NRM was the cause of 28% and 13% of deaths in years 3-5 after MAC and RIC, and 58% and 17% of deaths beyond 5 years, respectively. In particular, GVHD was the cause of 14% and 6% of late deaths after MAC and RIC (P=0.08) while second cancers were the cause of 12% and 2%, respectively (P=0.01). In all, 10-year NRM rate was 15%(11-20) and 9%(6-13), respectively (P=0.03). MVA identified RIC (HR 0.4, P=0.006), advanced disease at SCT (HR 2.3, P=0.03), age > 55 years (HR 1.8, P=0.07) and chronic GVHD (HR 2.0, P=0.03) as factors predicting for NRM.

**Conclusion:** Long-term LFS and GRFS are similar after RIC and MAC in AML. Pts who are leukemia free 2 years after SCT can expect good subsequent outcome with a 10-yr OS of 73%, after RIC or MAC. Disease status is the major predictor of subsequent OS. However, while relapse is the major cause of late death after both regimens, NRM and in particular GVHD and second cancers are more common causes of late death after MAC.

**Disclosure of Interest:** None declared.

0021

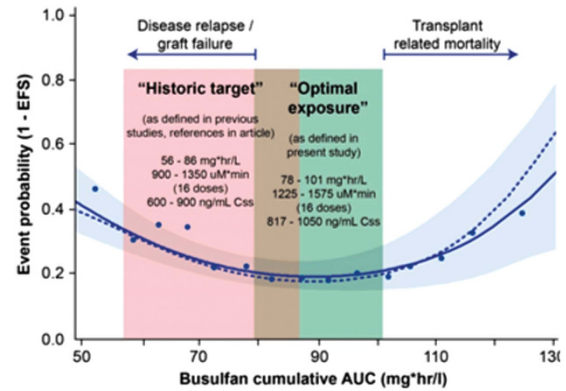
**Busulfan Exposure Predicts Event Free Survival and Toxicity after Hematopoietic Cell Transplantation in Children And Young Adults: A Multicenter Retrospective Cohort Analysis**

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**Introduction:** Intravenous-busulfan (IV-Bu) combined with therapeutic drug monitoring to guide dosing improves outcomes after hematopoietic cell transplantation (HCT). The best method to estimate Bu exposure and optimal exposure in children/young adults remains unclear. We therefore evaluated three approaches to estimate IV-Bu exposure and associated Bu-AUC with clinical outcomes in children/young adults undergoing HCT.

**Material (or patients) and methods:** Patients (0.1-30.4 years) who had received an IV-Bu conditioning regimen from 15 centers were included. Cumulative AUC was calculated by numerical integration using NONMEM (AUC<sub>NONMEM</sub>), non-compartmental analysis (AUC<sub>0-infinity</sub> and AUC<sub>0-tau</sub>) and by individual centers using a variety of approaches (AUC<sub>center</sub>). Primary outcome event-free survival (EFS) and other outcomes were modeled using propensity score adjusted cox



hazard, Weibull, and Fine-Gray competing risk regression models.

**Results:** 674 patients were included (41% malignant, 59% non-malignant) Estimated 2-year EFS was 69.7%. The median busulfan AUC<sub>NONMEM</sub> was 74.4 mg\*h/L (CI95% 31.1-104.6 mg\*h/L). The median AUC<sub>NONMEM</sub> correlated poorly with AUC<sub>center</sub> (R<sup>2</sup>=0.254). The Model of busulfan cumulative AUC in association with EFS in training (solid line) and validation dataset (dashed line) shows optimum of 78-101 mg\*h/L (Figure). Patients with optimal IV-busulfan AUC of 78-101 mg\*h/L showed 81% EFS at 2 years compared to 66.1% and 49.5% in the low (<78 mg\*h/L) and high (>101 mg\*h/L) busulfan AUC group (P=0.024), respectively. Graft-failure/relapse occurred more frequently in the low AUC group (HR=1.75 P<0.001). No difference in optimal exposure was observed among indications and HCT-source, or between 1, 2 or 3 alkylators in the conditioning regimen, but adding a 2nd or third alkylator did add toxicity. Acute toxicity, cGvHD and TRM was higher in the high AUC group (HR 1.69, 2.99 and 1.30), independent of indication.

**Conclusion:** These results demonstrate that Busulfan targeted using a validated pharmacokinetic-model to the optimal cumulative busulfan AUC of 78-101mg\*h/L, combined with fludarabine further optimizes the balance between efficacy and toxicity.

**Disclosure of Interest:** None declared.

0022

**Autologous stem cell transplantation for acute myelocytic leukemia in first remission : better outcome following busulfan and melphalan compared to busulfan and cyclophosphamide : a retrospective study**

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**Introduction:** Autologous stem cell transplantation for adult acute myelocytic leukemia still remains a therapeutic option for good and intermediate risk patients. We compared Busulfan + High dose Melphalan (BUMEL) to BUCY.

**Material (or patients) and methods:** From 2005 to 2013, 1073 patients received BUCY and 427 BUMEL. Since we found an interaction between the pretransplant regimen and the

interval from diagnosis to autografting, we studied the two groups separately.

**Results:** 1-Patients autografted within 6 months: 724 patients received BUCY and 225 BUMEL. In univariate analysis, patients autografted with BUMEL did better (figure) with a lower RI (34.3% vs 50.8%;  $P=0.0001$ ) a better LFS (61.8% vs 46%;  $P=0.0008$ ) and a better OS (79.2% vs 65%;  $P=0.005$ ). 60 patients received BUMEL and 238 BUCY at time when MRD detection was negative: The outcome post BUMEL was superior with a lower RI (28% vs 46.7%,  $P=0.02$ ) and a better LFS (69.8% vs 49.9%,  $P=0.02$ ). There also was a trend for a better OS (81% vs 68%;  $P=0.07$ ). By multivariate analysis (table), BUMEL compared with BUCY was significantly associated with lower RI, better LFS and OS.

	<i>P</i>	<i>HR</i>	<i>95% CI</i>	
<i>LFS</i>				
<b>BUMEL</b>	<b>.003</b>	<b>.67</b>	<b>.51</b>	<b>.87</b>
<b>Age</b>	<b>.001</b>	<b>1.15</b>	<b>1.06</b>	<b>1.26</b>
<b>Cytogenetics</b>	<b>.01</b>	<b>1.64</b>	<b>1.11</b>	<b>2.42</b>
<i>RI</i>				
<b>BUMEL</b>	<b>.001</b>	<b>.61</b>	<b>.46</b>	<b>.82</b>
<b>Age</b>	<b>.01</b>	<b>1.12</b>	<b>1.03</b>	<b>1.23</b>
<b>Cytogenetics</b>	<b>.01</b>	<b>1.71</b>	<b>1.14</b>	<b>2.56</b>
<i>OS</i>				
<b>BUMEL</b>	<b>.01</b>	<b>.63</b>	<b>.45</b>	<b>.89</b>
<b>Age</b>	<b>&lt; 10<sup>-5</sup></b>	<b>1.30</b>	<b>1.16</b>	<b>1.46</b>
<b>Cytogenetics</b>	<b>.06</b>	<b>1.53</b>	<b>.98</b>	<b>2.39</b>

2- 202 patients received BUMEL and 349 BUCY > 6 months after diagnosis: the outcome was similar.

**Conclusion:** We conclude that the BUMEL combination in CR1 better control AML than BUCY.

**Disclosure of Interest:** None declared.

**O023**

**Improved outcomes with a novel conditioning regimen in patients with thalassemia major who underwent hematopoietic stem cell transplantation from unrelated donors**

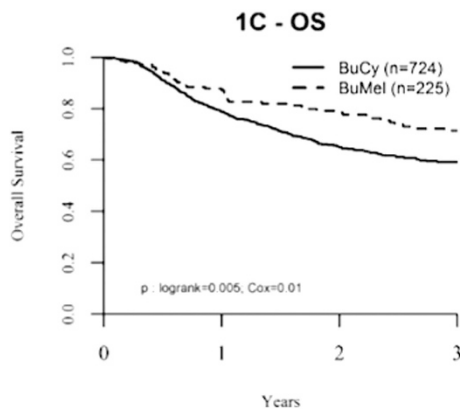
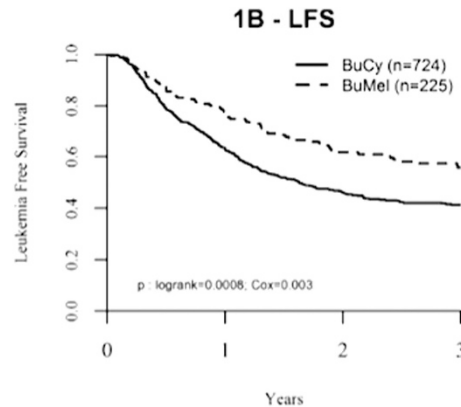
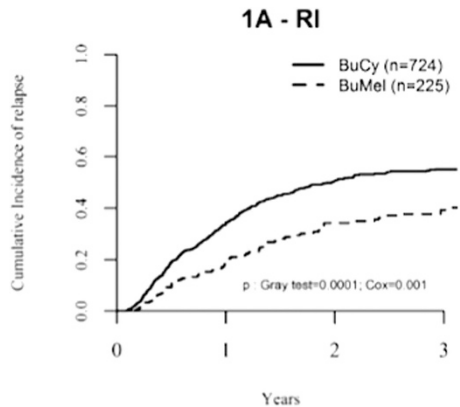
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**Introduction:** Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative option for patients with beta thalassemia major. However, the availability of HLA-matched related donor remains the main obstacle for allogeneic HSCT. Although, a few studies have been reported, experience with HLA matched unrelated donors is limited. We present the result of 15 children with beta thalassemia major who received allogeneic HSCT from HLA-matched unrelated donors with using a novel conditioning regimen.

**Material (or patients) and methods:** We retrospectively assessed 15 unrelated HSCT in children with beta thalassemia major. All patients received busulphan (BU) based myeloablative conditioning regimen. Busulphan was used according to weight adjusted doses. In addition, all patients received fludarabine 150 mg/m<sup>2</sup> in five days, cyclophosphamide 120 mg/kg in 3 days, thiotepa 10 mg/kg in one day and

[0022]



ATG 30 mg/kg in 3 days. Cyclosporin-A and MTX were used for graft versus host disease (GVHD) prophylaxis. Donor chimerism was evaluated in the peripheral blood on days +30, +100 and +180.

**Results:** The median age of the patients was 6 years (range 2-14 year). Two of the patients were grouped in Class I and 13 of them Class II. The median serum ferritin level was 1.186 ng/ml (range, 585-5832). All of the donors were matched 10/10 with high-resolution HLA typing in GVHD direction but two of them 9/10 with graft failure direction. Ten of them received BM (median TNC:  $7.30 \times 10^8$ /kg) and 5 PBSC (median MNC:  $7.43 \times 10^8$ /kg) with median CD34+ cell number  $7.20 \times 10^6$ /kg. The median neutrophil and platelet engraftment days were 11 and 16 days in PBSC and 13 and 19 days in BM group, respectively. Grade I-IV acute GVHD was observed in 4 patients (26%) and no chronic GVHD was seen. Moderate VOD was seen in 4 patients (26%) and treated with defibrotide successfully. All patients were alive with full donor chimerism (between 95.47-100 %) and followed up median 15 months (range 6-37 months).

**Conclusion:** These data show that the results of HSCT from unrelated donors in selected low risk thalassemia patients may be comparable to HSCT of matched sibling donors. However, it needs further studies with long term follow up and larger study population.

**Disclosure of Interest:** None declared.

#### O024

##### **NK Cells Characteristics and Anti-Tumor Ability In Multiple Myeloma Patients Before and After Autologous Stem Cell Transplantation**

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**Introduction:** Natural Killer (NK) cells are lymphocytes of the innate immunity with a potent anti-tumor capacity. In tumor patients (pts), such as multiple myeloma (MM) pts, an elevated number of NK cells correlates with a higher overall survival rate. We aimed to study NK cells characteristics and anti-tumor ability in tumor pts (16 MM, 16 lymphomas) before and after autologous stem cell transplantation (auto-SCT). Moreover, cytotoxicity of patient-derived, cytokine-stimulated NK cells against MM cells has been addressed at various time points (TPs) (at diagnosis, before/after auto-SCT).

**Material (or patients) and methods:** Pts' NK cells were analyzed by FACS after PBMCs isolation via Ficoll separation at different TPs: TP1, before high dose chemotherapy (HDC)/auto-SCT; TP2, after leukocyte recovery (leukocytes > 1000/ $\mu$ l) and TP3, at least 2 weeks after TP2. For testing NK cell cytotoxicity against MM cells, NK cells were purified via negative selection and expanded *in vitro* for 1-2 weeks in low doses IL-2 and IL-15.

**Results:** NK cells were divided into the CD56<sup>+</sup>CD16<sup>-</sup> or CD16<sup>+</sup> and CD56<sup>+</sup>CD16<sup>+</sup> subsets. While the major NK cell subset at TP1 was the CD56<sup>+</sup>CD16<sup>+</sup> subpopulation, after leukocyte recovery at TP2 CD56<sup>+</sup>CD16<sup>+</sup> cells were the main subsets. Surprisingly, CD57 expression was significantly increased within the CD56<sup>+</sup>CD16<sup>+</sup> population at TP2, but decreased again from TP2 to TP3. As expected, the NKG2A expression increased from TP1 to TP2 and remained elevated above the starting values until TP3. Interestingly, the KIR expression

within the CD56<sup>+</sup>CD16<sup>+</sup> cells was markedly increased at TP2 and remained elevated at TP3 in comparison to the starting values at TP1. More in details, both CD56<sup>+</sup> NK subsets upregulated their KIR2DL2/3/S2 and KIR3DL1 expression levels from TP1 to TP2, whereas the KIR2DL1/S1 levels remained stable. In addition, we evaluated NK cell functions upon tumor interaction at the three TPs. CD56<sup>+</sup>CD16<sup>-</sup> cells were the main subset to produce IFN- $\gamma$  upon interaction with K562 cells at all the TPs. The percentage of IFN- $\gamma$ -positive CD56<sup>+</sup>CD16<sup>-</sup> cells was slightly decreased at TP2 compared to TP1 but significantly increased from TP2 to TP3. Similarly, MIP-1 $\beta$ - and CD107a-positive CD56<sup>+</sup>CD16<sup>-</sup> cells remained constant between TP1 and TP2, whereas their percentages increased from TP2 to TP3. Moreover, in a small group of MM pts, we isolated NK cells and expanded them for 1-2 weeks prior to the functional assays. As expected, the expansion rate was reduced after chemotherapy but NK cells were still able to exert a residual killing of MM cells.

**Conclusion:** Our data demonstrate that NK cells have an altered phenotype after HDC/auto-SCT. Although the more "immature" CD56<sup>+</sup>CD16<sup>+</sup> subset was the main subset after leukocyte regeneration, it expressed increased levels of CD57 and KIRs, usually associated with a more mature phenotype. Remarkably, these NK cells could secrete cytokines and displayed a high cytotoxic capacity against different types of tumor cells. However, as the proliferative capacity of NK cells seemed to be reduced following chemotherapy, innovative NK cell therapeutic approaches might further improve the outcome of MM pts after auto-SCT.

**References:** Jacobs B, Tognarelli S, Poller K, Bader P, Mackensen A and Ullrich E (2015) NK Cell Subgroups, Phenotype, and Functions After Autologous Stem Cell Transplantation. *Front. Immunol.* 6:583. doi: 10.3389/fimmu.2015.00583.

**Disclosure of Interest:** None declared.

#### O025

##### **Matched pair comparison of concurrent cohorts of patients with relapsed/refractory (R/R) myeloma receiving an autologous stem-cell transplant (ASCT) using gemcitabine/busulfan/melphalan (GemBuMel) or single-agent melphalan**

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**Introduction:** Single-agent Mel is standard high-dose chemotherapy (HDC) for myeloma (MM). However, its results in R/R disease or as salvage ASCT are suboptimal.

**Material (or patients) and methods:** We have completed a phase 2 trial of a new HDC regimen of infusional gemcitabine (Gem), busulfan (Bu) and Mel (GemBuMel) for R/R myeloma.<sup>1</sup> Its scheduling optimizes Gem intracellular activation and exploits Gem inhibition of DNA damage repair. Eligibility included age 18-70, normal end-organ function, prior 1<sup>st</sup>-line treatment with bortezomib or an IMiD with either < PR or relapse, or receiving a salvage ASCT. We compared them with all other myeloma pts who were eligible for this study but instead received Mel off protocol at our institution during the same time period. We applied a matching algorithm to account for imbalances.

**Results:** 258 pts were analyzed, transplanted between 11/09-04/14 with GemBuMel (N=74) or Mel (N=184). The GemBuMel cohort had more pts with high-risk cytogenetics, double refractory tumors, unresponsive tumors at ASCT, or receiving a salvage ASCT. Both cohorts received similar post-ASCT maintenance (81% v 70%, P=0.1).



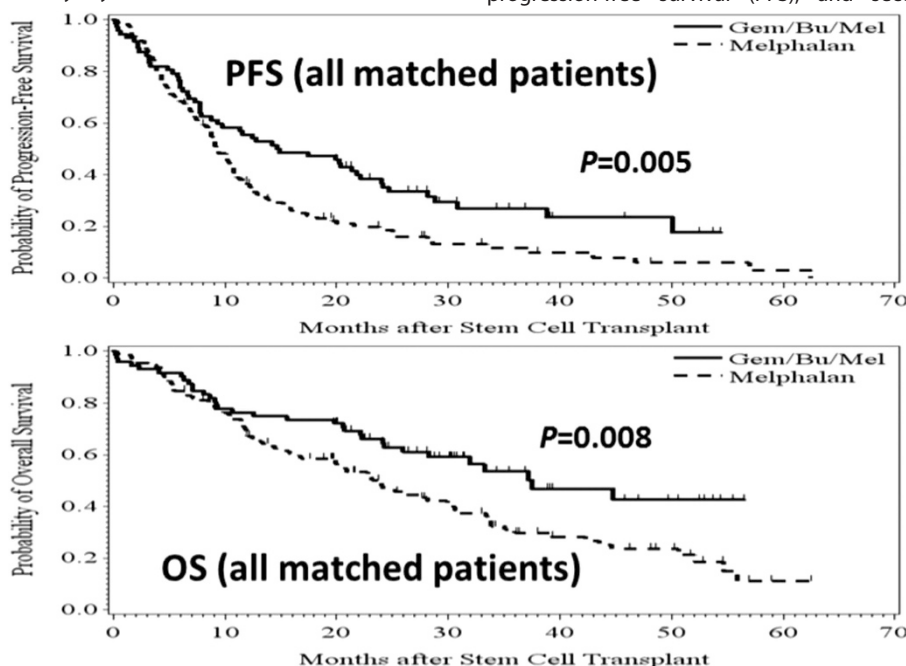
	GemBuMel (N = 74)	Mel (N = 184)	P
Age, median (range)	58 (22-68)	60 (31-70)	0.027
Poor-risk cytogenetics	51%	36%	<b>0.025</b>
Double refractory (IMiDs + PI)	76%	34%	<b>&lt; 0.001</b>
1 <sup>st</sup> SCT / Salvage SCT	57% / 43%	72% / 28%	<b>0.01</b>
Remission post 1 <sup>st</sup> SCT: ≥ 18 mo / < 18 mo	51% / 49%	58% / 42%	<b>0.6</b>
No. prior lines of treatment (IMWG criteria): 2 / > 2	53% / 47%	57% / 43%	0.58
N. prior relapses: 1 / > 1	61% / 39%	60% / 40%	1.0
Primary refr. / refr. rel. / sensit. rel / untreated rel	9% / 32% / 57% / 2%	5% / 22% / 53% / 20%	<b>0.0009</b>
Response at ASCT: Refractory / untreated	57% / 41% / 2%	53% / 27% / 20%	<b>&lt; 0.001</b>

Transplant-related mortality: 4% (GemBuMel) v 3.8% (Mel) ( $P=0.9$ ). In the GemBuMel and Mel cohorts sCR rates were 24.5% v 12.6% ( $P=0.04$ ), VGPR/sCR 44% v 38% ( $P=0.3$ ), and ORR 73% v 74% ( $P=0.7$ ). GemBuMel pts experienced longer PFS (median 14.8 v 9.3 months,  $P=0.005$ ) and lower risk of ogression/death (HR=0.64;  $P=0.043$ ). GemBuMel pts experienced longer OS (median 37.5 v 24.0 months,  $P=0.008$ ), with a lower risk of death (HR=0.61;  $P=0.043$ ). Within the groups receiving first ASCT, the GemBuMel cohort had improved PFS (median 19.9 v 10.1 months,  $P=0.008$ ) (HR 0.6,  $P=0.06$ ) and OS (median 44.8 v 25 months,  $P=0.007$ ) (HR=0.51,  $P=0.03$ ). Within the smaller subgroups receiving a salvage ASCT, the PFS and OS differences followed similar trends in favor of GemBuMel (PFS: median 15 v 9 months, HR=0.71,  $P=0.07$ ) (OS: median 34 v 24 months, HR=0.8,  $P=0.2$ ).

**Conclusion:** Despite their worse prognostic features, R/R MM pts treated with GemBuMel experienced improved PFS and OS as compared to a concurrent matched cohort receiving melphalan.

Reference: <sup>1</sup>Nieto Y et al. Gemcitabine/Busulfan/Melphalan with ASCT in Refractory Myeloma. Blood 122 (21), 2013.

[0025]



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**0026**

**Efficacy and Safety of Carfilzomib and Dexamethasone (Kd) Vs Bortezomib and Dexamethasone (Vd) in Patients With Relapsed Multiple Myeloma By Prior Autologous Stem Cells Transplantation: Secondary Analysis From the Phase 3 Endeavor Study (NCT01568866)**

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**Introduction:** Autologous stem cell transplantation (ASCT) is the standard of care for eligible multiple myeloma patients, but most patients eventually relapse. Here we report a subset analysis of patients with and without prior ASCT from the ENDEAVOR study.

**Material (or patients) and methods:** Patients with relapsed multiple myeloma (1-3 lines of therapy) were randomized 1:1 to Kd or Vd. Kd arm received carfilzomib (30-min IV infusion) on days 1, 2, 8, 9, 15, and 16 (20 mg/m<sup>2</sup> on days 1 and 2 of cycle 1; 56 mg/m<sup>2</sup> thereafter) and dexamethasone (d) 20 mg on days 1, 2, 8, 9, 15, 16, 22, and 23 of a 28-day cycle. Vd arm received bortezomib (btz) 1.3 mg/m<sup>2</sup> (IV or SC injection) on days 1, 2, 8 and 11 and d (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 of a 21-day cycle. We evaluated the primary endpoint, progression-free survival (PFS), and secondary endpoint,

overall response rate (ORR), as well as adverse events (AEs) in patients with and without prior ASCT.

**Results:** A total of 929 patients were randomized to Kd (n = 464) or Vd (n = 465); of these, 266 (Kd) and 272 (Vd) had a prior ASCT. Median age of patients and prior btz exposure were greater in the no prior ASCT group, but fewer patients were exposed to btz. Median PFS in the prior ASCT group has not been reached for Kd but was 10.2 months for Vd (hazard ratio [HR]: 0.61; 95% CI, 0.470-0.790). Median PFS in the no prior ASCT group was also longer for Kd vs Vd (17.7 vs 8.5 months; HR 0.43; 95% CI, 0.321-0.577). ORR was greater for Kd compared with Vd in both the prior and no prior ASCT groups. Grade  $\geq 3$  AEs were 72.2% (Kd) vs 63.8% (Vd) in the prior ASCT group and 74.6% (Kd) vs 71.3% (Vd) in the no prior ASCT group. Results are shown in the Table.

Table. Demographics, outcomes and AEs in patients with and without prior ASCT

	Prior ASCT		No Prior ASCT	
	Kd (n = 266)	Vd (n = 272)	Kd (n = 198)	Vd (n = 193)
Age, median years (range)	61.5 (35–79)	62 (30–76)	72 (39–89)	71 (44–88)
<i>Number of prior regimens, n (%)</i>				
1	123 (46.2)	141 (51.8)	109 (55.1)	91 (47.2)
$\geq 2$	143 (53.7)	131 (48.1)	89 (45.0)	102 (52.8)
Prior btz treatment, n (%)	167 (62.8)	163 (59.9)	83 (41.9)	89 (46.1)
Duration of prior btz, median months (min, max)	4 (0.1,38.0)	4.3 (0.3,27.4)	9.0 (1.3,46.0)	7.8 (1.0,33.0)
Median PFS, months	Not reached	10.2	17.7	8.5
HR for Kd vs Vd (95% CI)	0.61 (0.470–0.792)		0.43 (0.321–0.587)	
ORR, % (95% CI)	73.3 (67.6–78.5)	66.9 (61.0–72.5)	81.8 (75.7–86.9)	56.5 (49.2–63.6)
Grade $\geq 3$ AEs, % <sup>a</sup>	72.2	63.8	74.6	71.3

AE, adverse event; ASCT, autologous stem cell transplant; CI, confidence interval; HR, hazard ratio; Kd, carfilzomib and dexamethasone; btz, bortezomib; ORR, overall response rate; PFS, progression-free survival; Vd, bortezomib and dexamethasone. <sup>a</sup>The safety population included 266 (Kd) and 268 (Vd) in the prior ASCT group, and 197 (Kd) and 188 (Vd) in the no prior ASCT group.

**Conclusion:** Treatment with Kd resulted in prolonged progression-free survival compared with Vd and led to risk reduction of PFS events among patients with and without prior transplant. The use of Kd also led to higher response rates compared with Vd in patients with and without prior ASCT. These results suggest that Kd has a favorable benefit-risk profile and is a superior regimen over Vd for relapsed multiple myeloma regardless of transplant history.

**Disclosure of Interest:** M.-V. Mateos Conflict with: Honoraria of lectures and advisory boards from: Amgen, Celgene, Janssen, Novartis, BMS, Takeda, S. Knop Conflict with: Amgen: honoraria, J.-P. Femand: None declared, R. Hájek Conflict with: Celgene and Amgen: consultancy; Celgene, Amgen and Janssen-Cilag: honoraria, H. Ludwig Funding from: Takeda, Onyx, BMS, Janssen-Cilag: research funding, Conflict with: Takeda, Onyx, Celgene, Janssen-Cilag, BMS: honoraria; Takeda, Onyx, Celgene, Janssen-Cilag: Speakers Bureau, S. Feng Employee of: Onyx/Amgen, N. Mohamed Employee of: Onyx/Amgen, H. H. Gillenwater Employee of: Onyx/Amgen, K. Iskander Employee of: Onyx/Amgen, H. Goldschmidt Conflict with: Research Support: Celgene, Janssen, Chugai, Novartis, BMS, Millennium; advisory boards: Janssen, Celgene, Novartis, Onyx, Amgen Takeda, BMS; honoraria: Celgene, Janssen, Novartis, Chugai, Onyx, Millennium.

## O027

### Comparison of Haematopoietic Stem Cell Transplantation Approaches in Primary Plasma Cell Leukaemia

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**Introduction:** Primary plasma cell leukaemia is a rare and aggressive disorder. While autologous stem cell transplantation has been shown to improve outcome, response durations are short and further strategies are needed. Data is needed to help guide clinicians on the best approach to manage this disease. Therefore in an era of novel agents and improved autologous and allogeneic transplantation strategies this study compared different transplantation approaches in the treatment of PCL.

**Material (or patients) and methods:** A retrospective analysis was undertaken of the European Group for Bone Marrow Transplantation (EBMT) experience of patients with primary PCL undergoing haematopoietic stem cell transplantation between 1998 and 2012. Only patients who had achieved complete response, partial response or stable disease prior to transplantation were included. Patients with progressive or relapsed disease were excluded.

Data was collected using MED A and MED B forms. The primary end point was overall survival and data was analysed according to the information on the planned transplant strategy reported at the time of first transplant (intent-to-treat principle).

**Results:** A total of 460 patients were identified and categorised into 4 transplant groups.

Transplant approach	Number of patients
Single Auto	333
Double Auto	49
Auto-Allo <sup>1</sup>	20
Myeloablative	3
Reduced Intensity	16
Allo upfront <sup>2</sup>	58
Myeloablative	42
Reduced Intensity	12

1. One patient did not proceed to Allo.

2. Four patients not classified.

The follow up period ranged from 1 to 208 months with a median follow up of 48.9 months.

Patients undergoing allo upfront were found to have the worst overall survival. Compared to single auto this was statistically significant with a Hazard Ratio (HR) 1.85 ( $P=0.007$ ).

Compared to auto-allo the allo up front group had an even higher risk HR 3.18 ( $P=0.018$ ). The double auto versus single auto had a HR 1.39 which was not statistically significant ( $P=0.28$ ).

The auto-allo compared to single auto has a time varying effect with a higher mortality at the beginning and better risk afterwards. The effect on average is not significant HR 0.58 ( $P=0.24$ ).

When we consider time varying effects three periods were examined, 0-12, 12-36 and 36-60 months. Allo upfront is still confirmed to be inferior to single auto and also to auto-allo in the first year. Auto-allo has a trend to superiority versus single or double auto in the mid-term. However due to lack of follow up data in the auto-allo group it is difficult to assess possible benefits of auto-allo in the long term as there are few patients still at risk. Our initial analysis does suggest an advantage.

Preliminary analysis suggests that the auto-allo group enjoy the best progression free survival. The superiority of auto-allo versus allo upfront may be attributed to the higher rates of non-relapse related mortality observed in allo upfront.

**Conclusion:** Our preliminary work has demonstrated that allo upfront is associated with the worst overall survival. Further data is needed to reliably assess the outcome of auto-allo group which appears to be superior. Therefore we plan to request additional information from involved centres to strengthen the power of our results.

**Disclosure of Interest:** None declared.

#### O028

##### **The effect of body mass index and melphalan dose adjustments on outcomes in patients undergoing autologous haematopoietic cell transplantation for multiple myeloma: a single-centre retrospective study**

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**Introduction:** Despite the introduction of novel therapies, high-dose melphalan followed by autologous haematopoietic cell transplantation (AHCT) remains the standard of care for younger patients with multiple myeloma (MM). There are limited data on the effects of increased body mass index (BMI) on outcomes from AHCT and, in particular, whether or not melphalan dose adjustments should be made in patients with increased BMI. We therefore investigated the effect of increased BMI, and melphalan dose adjustments made for this, on outcomes from AHCT for MM.

**Material (or patients) and methods:** We conducted a retrospective study on all patients undergoing their first AHCT for MM from 2003-2013, and categorised them according to their BMI (normal weight: BMI 18.5-24.9, overweight: BMI 25.0-29.9, obese: BMI 30.0-34.9 and severely obese: BMI > 35.0). We investigated whether BMI group affected CD34+ cell collection, neutrophil/platelet engraftment, transplant-related mortality (TRM), progression-free (PFS) and overall survival (OS) rates. We also investigated, in a subgroup of patients with increased BMI, whether melphalan dose adjustments altered outcomes compared to those in whom no dose adjustments were made.

**Results:** 320 patients were included: 96 (30%) were normal weight, 143 (45%) overweight, 59 (18%) obese and 22 (7%) were severely obese. There were no significant differences between BMI groups in numbers of CD34+ cells harvested or transplanted. Neutrophil and platelet engraftment times were

also not significantly different. The 5-year OS rate was not significantly different between BMI groups (50.7% for normal weight, 60.9% for overweight, 57.2% for obese and 55% for severely obese,  $P=0.97$ ).

Patients were then divided into two groups according to scheduled melphalan dose (200mg/m<sup>2</sup>,  $n=223$ , and all other doses, predominantly 140mg/m<sup>2</sup>,  $n=97$ ). The median age of those scheduled for 200mg/m<sup>2</sup> was lower than for those scheduled to receive lower doses (57 versus 66 years,  $P<0.0001$ ), and a higher proportion of patients in the lower dose group had renal and cardiac co-morbidities. However, there was no significant difference in 2-year PFS (49% versus 52%,  $P=0.32$ ) or 5-year OS rates (61% versus 49%,  $P=0.26$ ) between these groups.

In 43 patients with BMI > 27.8 (33% of a total of 132), the melphalan dose was adjusted by the transplant physicians to 85-96% of that scheduled. Comparison of the dose-adjusted with the non-adjusted group showed no differences in age, sex or co-morbidities. There were no statistically significant differences between these groups in neutrophil (median 14.0 versus 13.0 days,  $P=0.11$ ) and platelet engraftment (median 16.0 versus 17.0 days,  $P=0.19$ ), 1-year TRM (0% versus 1%,  $P=0.81$ ), 2-year PFS (57.1% versus 47.8%,  $P=0.30$ ) and 5-year OS (61.2% versus 55.3%,  $P=0.84$ ).

**Conclusion:** In MM patients undergoing high-dose melphalan with AHCT, increased BMI does not alter CD34+ cell harvest, time to engraftment, 1-year TRM, 2-year PFS or 5-year OS. Melphalan dose reductions in patients with increased BMI do not appear to alter key transplant outcomes.

**Disclosure of Interest:** None declared.

#### O029

##### **Thalidomide maintenance, is it still effective in extending survival after autologous stem cell transplantation in patients with newly diagnosed multiple myeloma**

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**Introduction:** The efficacy of thalidomide maintenance has been reported in many studies. Whereas progression-free survival rates (PFS) improved, it remained controversial whether overall survival rates (OS) had improved after thalidomide maintenance in previous studies. However, there have been some concerns about the application of thalidomide maintenance in patients with MM eligible for ASCT in the real clinical field. The purpose of this study is to evaluate the utility of thalidomide maintenance in transplantation-eligible patients with MM in the real clinical field.

**Material (or patients) and methods:** Data from patients at 14 university hospitals in South Korea between June 2006 and April 2014 were collected retrospectively. All included patients had been treated with induction chemotherapy, followed by ASCT with or without thalidomide as maintenance therapy. Patients who had undergone allogeneic stem cell transplantation or tandem ASCT were excluded. The maintenance therapy with thalidomide was defined as a fixed oral dose of 50-100 mg thalidomide after ASCT. Additional steroids such as prednisolone or dexamethasone were permitted. Low-dose aspirin was also permitted to prevent thrombosis by physician's decision. When progression or relapse was recognized after ASCT, salvage chemotherapy was applied to the patients.

**Results:** The median age of the 258 patients was 57.5 years (range, 33-75 years) and the male to female ratio was 1.08:1.0. Standard cytogenetic risk was found in 197 patients (76.4%), intermediate risk in 40 (15.5%), and high risk in 21 (8.1%). One hundred one patients received maintenance therapy with thalidomide after ASCT, while the others did not. In 97 patients, progression or relapse was recognized after ASCT during follow-up periods, and only 82 patients received salvage chemotherapy. The median follow duration were 24.6 months. The median PFS and OS were 27.87 months (range, 20.1-35.6 months) and not reached, respectively. The 3-year PFS of patients treated with and without maintenance were 55.4% and 37.2% ( $P=0.005$ ). The 3-year OS of patients treated with and without maintenance were 88.0% and 84.0% ( $P=0.105$ ). The 3-year OS2 of patients treated with and without maintenance were 50.4% and 55.3% ( $P=0.661$ ). In particular, patients who showed less than CR after ASCT and had undergone maintenance therapy had superior survival rates to those who had not. Among the patients who showed less than CR after ASCT, the 3-year PFS with and without maintenance therapy were 68.4% and 23.3% ( $P<0.001$ ).

**Conclusion:** We have addressed several questions regarding the use of thalidomide maintenance therapy in real clinical practice. Thalidomide maintenance after ASCT may be helpful to prolong PFS in fit patients with MM. Long-term exposure to thalidomide in maintenance therapy may not affect survival (OS2) after relapse or progression from salvage chemotherapy. Finally, patients who have shown less than CR after ASCT may have the option of using thalidomide maintenance.

**Disclosure of Interest:** None declared.

#### O030

##### **A phase II study of bendamustine plus melphalan conditioning for second autologous stem cell transplantation in de novo multiple myeloma patients through a tandem transplant strategy**

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**Introduction:** This phase II trial evaluates, for the first time, the safety and efficacy of bendamustine combined with high dose melphalan (HDM) as conditioning regimen before the second autologous stem cell transplantation (ASCT) in previously untreated patients with multiple myeloma (MM).

**Material (or patients) and methods:** ASCT patients received HDM (200 mg/m<sup>2</sup>) as conditioning regimen before the first ASCT. After 3 to 6 months from the first ASCT, responding patients underwent a second ASCT following bendamustine (100 mg/m<sup>2</sup>) and HDM (140 mg/m<sup>2</sup>).

**Results:** Thirty-two patients (median age 56 years; 56% males) were enrolled. High-dose chemotherapy and ASCT were performed with complete neutrophil and platelet recovery in all patients. The median number of days to neutrophil and platelet engraftment were 11 (range 9-15) and 12 (range 10-19), respectively. Overall, the conditioning regimen was well tolerated. Only one subject experienced grade 3 diarrhea, and the rate of mucositis and vomiting was significantly lower with the bendamustine plus HDM regimen compared with the first transplant HDM-only regimen (81.2% vs 96.9%,  $P=0.025$  and 78.1% vs 100%,  $P=0.008$ ). ORR was 81.2% after the first transplant, and 90.6% after the second transplant. Actuarial 2-year PFS and OS were 79% (95% CI, 60 to 98) and 97% (95% CI, 91 to 100), respectively.

**Conclusion:** Bendamustine+HDM is feasible as conditioning regimen for second ASCT in patients with MM. The present study may pave the way for larger phase III studies specifically aimed at investigating safety, therapeutic activity and also the cost/efficacy ratio of this combination strategy.

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**Disclosure of Interest:** None declared.

#### O031

##### **The second autologous stem cell transplantation in treatment of multiple myeloma following conditioning based on treosulfan and melphalan: preliminary results from multicenter observational trial**

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**Introduction:** Autologous stem cell transplantation (autoSCT) remains the standard therapy incorporated in the first line strategy for patients (pts) with multiple myeloma (MM). Despite numerous modern treatment option for MM, the effective salvage therapy is lacking. One of the possibilities in the treatment of pts relapsing after autoSCT is the second autologous transplant, however considering resistance at relapse and increased risk for toxicity of second procedure, conditioning regimen seems to be an issue. Based on *in vitro* studies treosulfan, which strongly induces apoptosis of myeloma cells, can be used as a part of conditioning regimen for MM, especially safety of its combination with melphalan was confirmed in pediatric setting. Therefore, we analyzed the outcomes of MM pts undergoing the second autoSCT following conditioning with treosulfan+melphalan with respect to safety of the treatment and durability of response.

**Material (or patients) and methods:** Thirty patients with median age of 55 years (range: 40-65), who relapsed after prior autoSCT were analyzed. All pts for the first transplant were conditioned with melphalan 200 mg/m<sup>2</sup>. The study cohort included pts with stage II (17) and III (7) according to ISS. The median time between the first autoSCT and relapse was 11 months (range: 3-48) and the median time between the first and the second autoSCT was 20 months (range:6-87). Eleven pts relapsed within 6 months after the first autoSCT. Before the second autoSCT, 26 pts were at least in partial remission. For conditioning before the second transplant all pts received treosulfan 10 g/m<sup>2</sup> on days -5, -4, -3 and melphalan 140 mg/m<sup>2</sup> on day -2. Peripheral blood stem cells were grafted in median dose 4.2 (2.8-10.5)x10<sup>6</sup> of CD34+ cells/kg.

**Results:** Neutrophil and platelet reconstitution occurred in all transplanted pts at median 13 days (range: 9-22) and 15 days (range: 9-28), respectively. Infectious complications were observed in 15 (50%) pts during neutropenia (febrile of unknown origin 11, bacterial 3, fungal 1). Late infections occurred in 6 (20%) pts, and were related to bacterial (2) or viral (Herpes zoster) infection. Severe toxic organ complications (CTCAE grade 3-4) were not seen. In 2 pts prolonged vomiting and nausea were observed and in 3 pts mucositis grade 3 according to WHO had occurred. Seventeen pts

experienced disease relapse after the second transplant in median 7 months (range: 3-17). Over a median follow-up of 15 months (range: 2-49) 24 (80%) pts are alive. In 4 cases deaths were related to relapse, 1 pt died due to infection complications and 1 due to secondary neoplasm. For whole group probability of 3-years overall survival (OS) and disease free survival (DFS) were  $0.614 \pm 0.136$  and  $0.206 \pm 0.10$ , respectively. In subgroup of pts who relapsed >6 months after first autoSCT, 1 year DFS was statistically longer than in those relapsing  $\leq 6$  months:  $0.75 \pm 0.11$  vs  $0.22 \pm 0.14$ ;  $P = 0.003$ , but it has no impact on OS.

**Conclusion:** The second autoSCT is a salvage option for relapsing MM patients. The analysis confirms that treosulfan + melphalan is a safe, low toxicity conditioning regimen and produces durable responses in patients with late relapse.

**Disclosure of Interest:** None declared.

### O032

#### A prospective randomized study comparing tacrolimus and sirolimus versus cyclosporine and methotrexate as gvhd prophylaxis in allogeneic hematopoietic stem cell transplantation

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**Introduction:** Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment for a series of otherwise lethal hematopoietic disorders. Despite continuous refinements of transplant procedures, graft-versus-host disease (GVHD) remains a serious complication after treatment, significantly contributing to morbidity and mortality. Hence, improvement of GVHD prophylaxis remains an important goal in HSCT. Based on previous reports of possibly preferential properties of sirolimus, we compared a combination of tacrolimus and sirolimus with cyclosporine and methotrexate as GVHD prophylaxis after HSCT in this prospective, open, randomized trial.

**Material (or patients) and methods:** We assessed 532 consecutive patients accepted for treatment with HSCT at two transplant centers between 2007 and 2014. According to study criteria, 215 patients were enrolled in the study and randomized at a ratio of 1:1 to GVHD prophylaxis with either tacrolimus and sirolimus ( $n=106$ ) or cyclosporine and methotrexate ( $n=103$ ). The primary endpoint was acute GVHD of grades II-IV in the two groups within 3 months after HSCT. Secondary endpoints included time to neutrophil and platelet engraftment, incidence of acute GVHD grades III-IV, chronic GVHD, incidence of oropharyngeal mucositis, treatment-related toxicities, infections, disease relapse, transplant-related mortality and overall survival.

**Results:** There was no significant difference in the cumulative incidence of acute GVHD of grades II-IV (51% vs. 41%;  $P=0.19$ ) or acute GVHD of grades III-IV (7% vs. 13%;  $P=0.09$ ) between the two groups. Time to neutrophil engraftment was similar (17 days vs. 18 days;  $P=0.24$ ), but time to platelet engraftment was shorter in the tacrolimus/sirolimus group (12 vs. 14 days;  $P<0.01$ ). No significant differences in incidence of oropharyngeal mucositis, time to full donor chimerism or number of CMV infections were seen between the two treatment arms. Distribution of transplant related toxicities was equal in both study groups, but a suspected increase in VOD and TMA in

patients receiving tacrolimus/sirolimus after myeloablative conditioning with busulphan and cyclophosphamide (BuCy) was noted. This finding led to the decision to stop further recruitment of patients receiving BuCy conditioning to the trial. Transplant-related mortality (12% vs. 18%;  $P=0.40$ ) and five-year overall survival (71% vs. 72%;  $P=0.71$ ) were similar. Five-year relapse-free survival in the subgroup of patients with malignant diagnoses was 63% in the tacrolimus/sirolimus group and 65% in the cyclosporine/methotrexate group ( $P=0.73$ ).

**Conclusion:** We did not find any significant advantages of tacrolimus/sirolimus over cyclosporine/methotrexate as GVHD prophylaxis after HSCT in this prospective randomized trial, despite the inclusion of a relatively heterogenic HSCT-patient population. Still, our results confirm that tacrolimus/sirolimus is a valid and safe GVHD prophylaxis option after HSCT, with transplant-related outcomes comparable to those with cyclosporine and methotrexate.

**Disclosure of Interest:** None declared.

### O033

#### Pentraxin 3 as a Biomarker for Acute GvHD and Fungal Infections in Allogeneic Transplantation: Results of a Prospective Longitudinal Study in 73 Adult Recipients

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**Introduction:** Pentraxin3 (PTX3) is an essential component of the humoral arm of innate immunity, involved in controlling infections, modulating inflammation, and acting as a new prognostic and diagnostic biomarker in these conditions. PTX3 has been recently recognized as an extrinsic oncosuppressor in cancer; its role in hematological malignancies and allogeneic HSCT (allo-HSCT) is under investigation in both pediatric and adult settings.

**Material (or patients) and methods:** From 2012 to 2014, we conducted a prospective observational study to address PTX3 role in 35 adult patients (pts) affected by newly diagnosed AML ( $n=27$ ) or ALL (8), and in 73 adult pts who received allo-HSCT for acute leukemia. Donors were haploidentical ( $n=45$ ), sibling ( $n=14$ ), unrelated ( $n=14$ ). Serial peripheral blood (PB) and bone marrow (BM) samples were collected. Time-points of sampling for leukemia pts included: diagnosis, relapse, any cycle of CT, evaluation of response (including BM), fever. Samples from allo-HSCT pts were collected as following: before HSCT, at HSCT (day 0), day +7, day +14, 1 month (including BM), 2 months (including BM), 3 months (including BM), 6 months (including BM), fever, acute GvHD requiring systemic therapy (at onset or before second line, day +7 of treatment, then weekly for 6 weeks). PTX3 level detection was performed with sandwich ELISA (detection limit 0.1 ng/ml).

**Results:** Firstly we analyzed the group of leukemia pts. Although PTX3 levels on PB at diagnosis were not influenced by pts (age, sex) or disease characteristics (type of leukemia, BM and PB blasts), they correlated with WBC counts ( $P=0.03$ ). No statistical difference was found matching PTX3 at diagnosis with relapse or overall survival (OS).

Then examining allo-HSCT pts, we observed an important difference in PTX3 levels on PB at day 0 among pts treated with treosulfan or busulfan-based conditioning ( $P=0.027$ ). We found a trend towards an increase of PTX3 for pts who developed a subsequent GvHD at day +7, and for pts who received ATG instead of PT-Cy at day +14 ( $P=0.06$  for both).

Moreover, we evaluated 38 events of acute GvHD. Interestingly, PTX3 on PB at GvHD onset predict a subsequent evolution to chronic GvHD ( $P=0,02$ ), also confirmed by ROC analysis (AUC 0.786) which allowed us to identify a threshold of 58 ng/ml (sens 75%, spec 85%). At day +7 from the start of therapy, PTX3 levels were correlated directly with steroid-refractory GvHD ( $P=0,004$ ) and reflare ( $P=0,024$ ), and inversely with GvHD resolution ( $P=0,003$ ). Concomitantly, the ROC analysis identified a threshold of 18 ng/ml as predictor for steroid-resistance (AUC 0,91; sens 75%, spec 85%) and GvHD resolution (AUC 0,848; sens 70%, spec 85%). Finally, we reported 142 infectious events. No difference in PTX3 was reported for first and subsequent episodes, or with bloodstream infections. Importantly, in presence of invasive fungal infection (IFI), the levels of PTX3 were significantly increased ( $P=0,016$ ).

**Conclusion:** Our results demonstrate that PTX3 acts as early biomarker for major transplant-related complications, as IFI and acute GvHD, still leading causes of death after HSCT. This study suggests that PTX3 is able to predict GvHD resolution and the risk of subsequent chronic GVHD and significantly steroid-refractory GvHD, providing new insight into the pathogenesis of the syndrome, and facilitating advances in clinical management.

**Disclosure of Interest:** None declared.

#### O034

##### **Atorvastatin is effective for graft versus host disease prophylaxis - Results of a phase II prospective trial**

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**Introduction:** Atorvastatin (ATOR) administration to both donors and recipients of matched related donor (MRD) alloHCT is safe and effective for aGVHD prophylaxis (REF1). We conducted a multicenter, phase II study (NCT01665677) to evaluate the safety and efficacy of ATOR-based aGVHD prophylaxis given only to the recipients of MRD and or unrelated (MUD) alloHCT.

**Material (or patients) and methods:** Between Sep 2012 and March 2015, we enrolled 69 patients receiving alloHCT from MRD ( $n=30$ ) or MUD ( $n=39$ ). All patients were 10/10 (A/B/C/DR/DP) HLA matched and received peripheral blood cell allografts. Acute GVHD prophylaxis consisted of tacrolimus, micro-dose methotrexate and ATOR (40mg/day) from days -14 to +180. Graft manipulation and T cell depletion were not permitted. The study was powered to detect a 20% reduction in day 100 Gr 2-4 aGVHD relative to historical rates (35% to 15% in MRD and 55% to 35% in MUD).

**Results:** Table 1 shows baseline pt characteristics. The only protocol related Gr 3 non-hematologic toxicity was ALT elevation in one MRD. All pts engrafted (median of 16 days for MRD and 15 days for MUD grafts). Median day 100 CD33+ cell chimerism in both cohorts was 100%. The cumulative incidence (CI) of Gr 2-4 and Gr 3-4 aGVHD at day100 in the MRD cohort was 9.9% (95%CI: 0-20%) & 3.4% (95%CI: 0-9.7%) and in MUD cohort 29.8% (95%CI: 15.7-43.9%) & 18.3% (95%CI: 6.2-30.3%), respectively. CI of Gr 2-4 aGVHD at day180 were 16% and 34.9% in MRD and MUD, respectively. The respective figures for Gr 3-4 aGVHD at day180 were 3.4% and 21.4%. CI of moderate/severe chronic GVHD at 1 year for MRD and MUD were 35.6% (95%CI: 15.7-55.4%) and 43.5% (95%CI: 23.1-63.1%), respectively. At 1-year, the rates of non-relapse mortality were 3.3% and 9.4%; relapse 43% and 19%; progression-free survival 50% and 71%, and overall survival 65% and 76%, respectively for MRD and MUD cohorts.

Table 1.

	MRD N = 30 (range)	MUD N = 39 (range)
Male pts	17	27
Median age, yrs	61 (19-72)	58 (23-74)
Median days on ATOR	183 (26-533)	141 (22-202)
Median HCT-CI	2 (0-7)	2 (0-5)
<i>CIBMTR Disease Risk</i>		
Low	12	22
Intermediate	9	5
Advanced	9	12
<i>Diagnosis</i>		
Leukemia/MDS	36	20
Lymphoma	3	8
Others	-	2
Chemorefractory	9	12
<i>Conditioning</i>		
RIC	17	15
Myeloablative	13	24
<b>Median Follow-up of survivors, days</b>	<b>378 (245-973)</b>	<b>299 (176-720)</b>

**Conclusion:** Addition of ATOR to standard GVHD prophylaxis in MRD and MUD HCT appears to be feasible, safe and effective in reducing aGVHD risk.

Reference: 1. Hamadani et al. JCO. 2013;31:4416-23.

**Disclosure of Interest:** None declared.

#### O035

##### **An International, Phase III, Randomized, Multicenter, Parallel-group Study of Inolimomab vs. usual care for the Treatment of Primary Steroid Refractory Acute Graft Versus Host Disease (aGVHD) Following Allogeneic Stem Cell Transplantation in Adults**

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**Introduction:** Acute graft versus host disease (aGVHD) is a potentially fatal complication of SCT that occurs in 30% to 80% of patients. High-dose corticosteroids are first-line treatment for aGVHD; however, up to 50% of patients fail to obtain a satisfactory response with steroid treatment alone. Treatment of steroid-refractory aGVHD remains an unmet clinical need. Although numerous phase II clinical trials have been reported, to date only 1 randomized trial has been published in this setting\*. Inolimomab, a monoclonal antibody to CD25 of the interleukin-2 receptor, has shown encouraging results in a phase II trial (INO-0102) as salvage therapy for patients with steroid-refractory aGVHD. The goal of this study was to

compare inolimomab treatment to usual care in patients presenting with steroid-refractory aGVHD.

**Material (or patients) and methods:** This phase III, randomized, open-label, multicenter trial aimed to compare inolimomab vs. usual care in adult patients ( $\geq 18$  years) with steroid-refractory aGVHD. The study was conducted at 11 centers in France and Belgium. Patients were randomized to treatment with inolimomab or usual care. Inolimomab-treated patients received 0.3 mg/kg/day during induction and 0.4 mg/kg 3 times per week during maintenance. The total treatment duration was  $28 \pm 2$  days and patients were followed for up to 12 months. Patients in the control group were all treated according to the usual care (including high dose of steroids, Anti Thymocyte Globulin [ATG], TNF $\alpha$  blockers, and other chemotherapy). The primary objective of this study was to evaluate overall survival (OS) at 1 year without replacement of baseline allocated therapy.

**Results:** A total of 100 patients were randomized; 49 patients in the inolimomab arm and 51 patients in the usual care arm (all treated with ATG). The arms were well balanced with regard to gender, age, conditioning regimen, and aGVHD status. The primary criteria (1-year survival without replacement of baseline allocated therapy) was reached by 14 patients (28,5%) in inolimomab and 11 patients (21,5%) in the usual care (ATG) arm, with a hazard ratio of 0,874 ( $P=0,2804$ ). Figure: Time from randomization to treatment failure (defined as death or change of baseline treatment regimen).

With a minimum follow-up of 1 year, 26 (53%) and 31 (60%) patients died in the inolimomab and the usual care (ATG) arms, respectively. The number of adverse events were similar in the two arms (96% vs 90 % with at least one SAE), with fewer viral infections in the inolimomab arm (38;78%) compared to the usual care (ATG) arm (47;92%).

**Conclusion:** Although the primary endpoint of this randomized phase III trial was not achieved, these results showed a trend towards improved 1-year OS with inolimomab compared to usual care (ATG). The lack of a statistically significant effect confirms the need for development of more effective treatments for aGVHD.

**References:** \*MacMillan et al. *Blood* 2007;109:2657-2662.

**Disclosure of Interest:** G. Socié Funding from: Jazz Pharmaceuticals, S. Vigouroux: None declared, I. Yakoub-Agha Funding from: Jazz Pharmaceuticals, J.-O. Bay Conflict with: Jazz Pharmaceuticals, S. Fürst: None declared, K. Bilger: None declared, F. Suarez Conflict with: Jazz Pharmaceuticals, M. Michallet Funding from: Jazz Pharmaceuticals, D. Bron: None declared, Z. Medeghri Employee of: Jazz Pharmaceuticals, P. Gard Employee of: Jazz Pharmaceuticals, P. Lehert Funding from: Jazz Pharmaceuticals, C. Lai Employee of: Jazz

Pharmaceuticals, T. Corn Employee of: Jazz Pharmaceuticals, J.-P. Vernant: None declared.

### O036

#### It is EASIX to predict death from acute GVHD

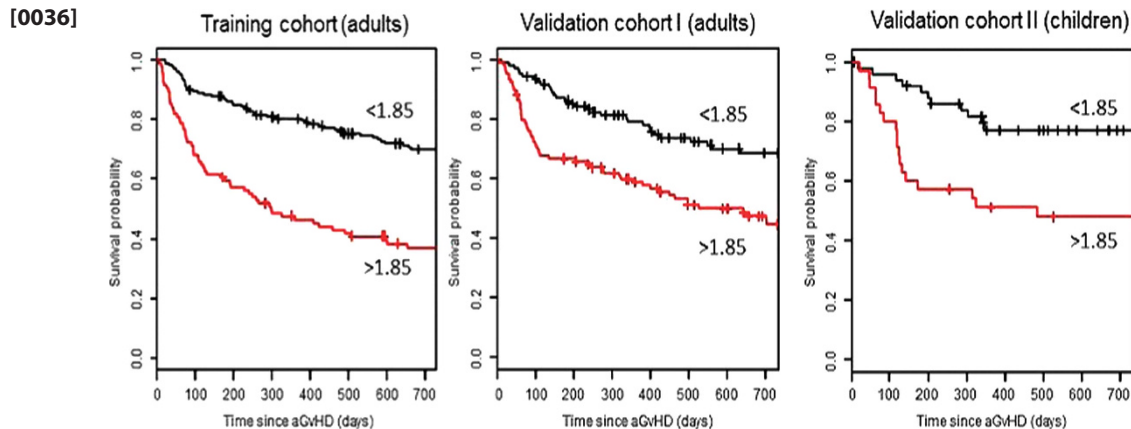
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**Introduction:** Recent pre-clinical and clinical data identified endothelial cell dysfunction as a major contributor to refractory GVHD and death. Thrombotic microangiopathy (TMA) and refractory acute graft-versus-host disease (refrGVHD) are lethal complications after allogeneic SCT (alloSCT). Both conditions seem to be associated with endothelial damage and in our cohort overlap in about 50% of cases. Based on the fact that renal TMA is defined by high creatinine, high lactate dehydrogenase (LDH) and low thrombocyte counts, with schistocytes and loss of haptoglobin as additional markers, we hypothesized that the simplified formula 'creatinine times LDH divided by thrombocyte counts' (endothelial activation and stress index, EASIX) might be valuable for predicting TMA and the related complication refractory GVHD.

**Material (or patients) and methods:** The capacity of EASIX raised on day 0 of alloSCT to predict TMA was tested retrospectively in 771 consecutive adult patients undergoing alloSCT in Heidelberg between 2001 and 2013 using cause-specific Cox regression. Moreover, EASIX was tested as predictor of acute GVHD outcome when measured at GVHD onset in a subset of 273 Heidelberg patients experiencing this complication. The prognostic capacity of the EASIX model for GVHD mortality was validated in two independent cohorts ( $n=86$  children, Cincinnati;  $n=220$  adults, Berlin) by calculating the prediction error (integrated Brier score), concordance index, and calibration index.

**Results:** EASIX significantly predicted TMA when measured on day 0 of alloSCT (HR=1.20 (increase by log2 steps)  $P=0.03$ ), and even if the marker was assessed prior to conditioning (HR=1.24,  $P=0.03$ ). EASIX was also a significant prognostic factor for TMA in multivariable models including age, disease score, ATG, donor sex, recipient sex, graft source, diagnosis and statin intake as covariates (d0, HR 1.41,  $P < 0.001$ ; pre-conditioning, HR 1.28,  $P=0.02$ ). In patients with acute GVHD,



**Figure 1:**

OS after acute GVHD onset in three independent cohorts according to EASIX:

EASIX assessed at GVHD onset strongly predicted overall survival (OS) in univariable (HR=1.18,  $P < 0.001$ ) and multivariable (HR 1.18,  $P = 0.001$ ) models. Model validation for EASIX at acute GVHD onset was successfully done in the two independent cohorts. In order to visualize the clinical value of assessing EASIX at acute GVHD, optimal cut-offs were calculated using maximally selected logrank statistics for data of the Heidelberg cohort. A highly significant cut-off could be derived at EASIX = 1.85. OS since acute GvHD according to the cut-off is shown for each cohort separately in Figure 1.

**Conclusion:** The novel, extremely easy-to-assess EASIX score is a powerful predictor of mortality after acute GVHD, warranting further prospective validation.

**Disclosure of Interest:** None declared.

### O037

#### An Open Label Randomized Phase 2 Trial of Topical Dexamethasone and Tacrolimus Solutions for the Treatment of Oral Chronic Graft-Versus-Host Disease

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**Introduction:** The objective of this study was to evaluate the safety and efficacy of single agent dexamethasone (DEX) or tacrolimus (TAC) topical solution for the management of new onset symptomatic oral chronic graft-versus-host disease (cGVHD), and to determine if one therapy is superior.

**Material (or patients) and methods:** This was a prospective single-center open label randomized phase II trial of patients with new onset oral cGVHD without prior topical, and with stable systemic immunomodulatory therapy. Subjects were randomly assigned 1:1 to either topical DEX (0.5 mg/mL) or TAC (0.5 mg/mL) solution and instructed to rinse with 5 mL for five minutes, four times a day, for four weeks. Oral cGVHD assessments (NIH criteria) were completed at baseline and end of treatment (NIH criteria, global response, and tolerability). The primary endpoint was the response rate defined as  $\geq 3$  point reduction from pre to post treatment with respect to sensitivity score (0-10). Subjects who received new immunomodulatory medications, dose adjustments, or initiated ECP during the treatment period were not evaluable for response. Since DEX was not systematically evaluated in a randomized setting, a parallel two-stage design was employed instead of a direct comparison in efficacy, so that an arm could be terminated early in the event of inefficacy. The accrual goal was 60 evaluable patients; 30 in each arm, accruing 14 in the first stage and 16 in the second stage. In the first stage, if 7 or fewer patients achieved a response, accrual to the arm would be stopped, otherwise accrual would continue for an additional 16 patients. If 11 or more responses were observed among the 30 total evaluable patients in each arm, then the treatment would be considered efficacious. If both arms were regarded as efficacious, a 'pick-the-winner' method would be employed to choose a better treatment for future investigation.

**Results:** Forty six subjects were randomized to receive either DEX ( $n = 28$ ) or TAC ( $n = 18$ ). Six subjects were excluded from the analysis due to changes in systemic immunosuppression (DEX=1, TAC=3), or lack of second follow-up (1 per arm). After the first stage evaluation, the TAC arm was terminated due to lack of activity (3/14 responses, response rate 21%). Twenty six subjects in the DEX arm completed both study visits and were included in the response analysis, with 58% (15/26) experiencing response. With respect to how their mouths overall felt since beginning treatment, 31% vs. 21% patients reported "much better", and 38% vs. 36% reported "slightly better", giving an overall global response rate ("much better" or "slightly better") of 81% (21/26) vs. 71% (10/14), in the DEX and TAC arms, respectively. DEX rinses were well

tolerated and taste was reported as "very pleasant" or "tolerable" in most subjects (96%).

**Conclusion:** Intensive topical therapy with DEX solution is effective for managing patients with new onset symptomatic oral cGVHD and should be considered for first-line therapy. Topical TAC solution is ineffective and is not indicated for first-line therapy.

**Disclosure of Interest:** None declared.

### O038

#### Phase I-II Study of Calcineurin and M-tor Inhibitor-Free Post-transplant Cyclophosphamide and Bortezomib (CyBor) for the Prevention of Graft-Versus-Host Disease

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**Introduction:** Current graft-versus-host disease (GVHD) prophylactic regimens based solely on T cell suppression remain unsatisfactory. Besides selectively depleting alloreactive T cells and promoting the emergence of suppressor T cells, bortezomib (Bor) inhibits dendritic cell maturation and function.

**Material (or patients) and methods:** In a phase I-II study, we sought to explore the feasibility and efficacy of adding of Bor to a platform of post-transplant cyclophosphamide (Cy) in patients undergoing allogeneic peripheral blood hematopoietic stem cell transplantation from 8 out 8 allele-matched siblings or volunteer donors. To date, 25 patients have been enrolled. The conditioning regimen was based on fludarabine (total 180 mg/m<sup>2</sup>) and busulfan (total 6.4 mg/kg). Patients receiving grafts from unrelated donors ( $n = 15$ ) also received rabbit anti-thymocyte globulin (Thymoglobulin<sup>®</sup>) 8 mg/kg in the first 4 patients and 5 mg/kg thereafter. GVHD prophylaxis consisted of Cy 50 mg/kg given on days +3 and +4 and Bor. Bor was given 6 hours after graft infusion and 72 hours thereafter. The dose was escalated as follows: 0.7, 1, and 1.3 mg/m<sup>2</sup> in 3 cohorts ( $n = 3, 3, \text{ and } 19$ ).

**Results:** Median age was 57 years (37-70). Ten patients had AML, 6 MDS, 4 CLL, 2 DLBCL, 1 ALL, 1 follicular NHL, and 1 MM. Three patients had decreased creatinine clearance (50-60 mL/min/1.73m<sup>2</sup>) at enrollment. One patient is not yet evaluable for engraftment. In the remaining patients, median time to neutrophil engraftment was 16 days (13-23). Two patients died before meeting criteria for platelet engraftment. In the remaining 22 patients, median time to platelet engraftment was 27 days (15-38). With the exception of 1 patient, all evaluable patients achieved full chimerism by day +24. The remaining patient had residual CLL and reached full chimerism by day +118. There was no secondary graft failure. No other than hematologic grade  $\geq 3$  toxicity was encountered. Treatment-related mortality was 12.5%. Two patients developed extensive HSV genito-rectal ulcers. No other cases were encountered when institutional practice changed to start acyclovir on the first day of conditioning instead of day +5. CMV and EBV reactivation occurred in 13 & 5 patients with one patient developing post-transplant lymphoproliferative disease. Three patients developed BK virus-associated hematuria and 1 CNS toxoplasmosis. Immune reconstitution data was available on 16 patients (Figure 1). With a median follow up of 17.6 m (0.7-30), 9 patients (37.5%) relapsed: 2 DLBCL, 4 AML, 1 ALL, 1 MDS, and 1 MM. Out of 23 patients evaluable for acute GVHD, 7 patients developed grade II-IV (30.4%) and 3 (13%) grade III-IV. Three out of 19 patients with  $> 6$  months follow-up (15.8%) developed chronic GVHD. The predicted 2-year DFS and OS were 53.1% and 56.9% (Figure 2 A) while the predicted GVHD and relapse-free survival (GRFS) was 37.1%. The predicted 2-year OS for patients with Pre-Assessment Mortality (PAM) scores  $< 19$  ( $n = 11$ ) and  $> 19$  ( $n = 13$ ) was 81.8% and 25.5% ( $P$  value 0.024) (Figure 2 B).

**Conclusion:** Despite the small size, our study suggests that post-transplant CyBor is safe and practical to use even in patients with mild to moderate renal failure. It also allows



[0038]

Figure 1

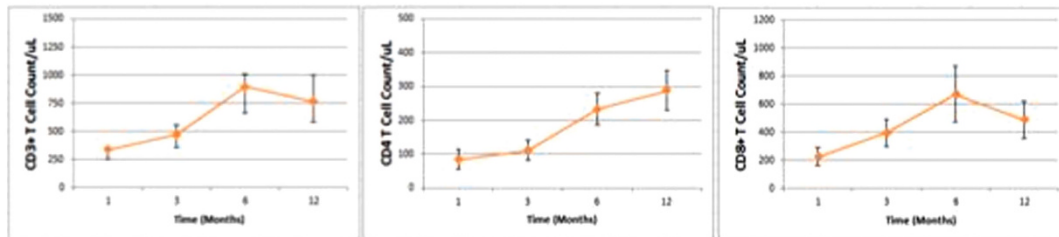
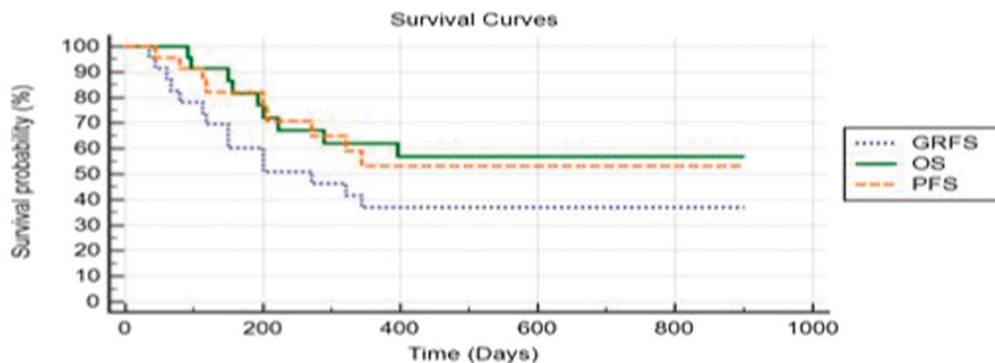
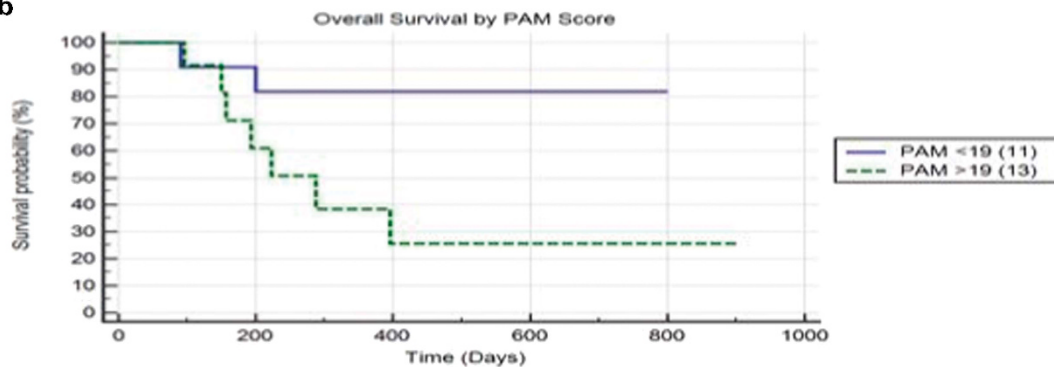


Figure 2

a



b



prompt immune reconstitution and yields favorable outcomes. CyBor merits further investigation in a larger multi-institutional trial.

**Disclosure of Interest:** A. S. Al-Homsi Funding from: Millennium Pharmaceuticals, K. Cole: None declared, M. Muilenburg: None declared, U. Duffner: None declared, M. Abidi: None declared, S. Williams: None declared, A. Mageed: None declared.

O039

**Sclero-corneal lenses safe and efficient for the treatment of keratoconjunctivitis sicca in patients with refractory ocular gvhd: a study on behalf of the SFGM-TC**

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**Introduction:** Keratoconjunctivitis sicca syndrome (KCS) due to chronic GvHD (cGvHD) is responsible for major alteration in quality of life of patients undergoing allogeneic stem cell transplantation (allo-CST). The conjunctival fibrosis secondary to inflammation is often slow and subtle and is responsible for impaired corneal and conjunctival epithelial surfaces potentiated by tear quantitative and qualitative deficiency. Treatment of KCS remains disappointing; variable success in the correction of the conjunctival dryness has been obtained with variety of topical treatments with or without systemic immunosuppressive agents. These treatments, though, do not seem to have any effect on sicca symptoms and patients' quality of life. Sclero-corneal lenses bring a valid therapeutic option by creating a pre-corneal reservoir of tears allowing permanent lubrication of the epithelium, the protection of the corneal surface against the eyelid and ciliary mechanical friction and against environmental stresses and the creation of a uniform refractive surface to be taken into optimum optical load and stable visual acuity.

**Material (or patients) and methods:** We describe the safety and efficacy of Sclero-corneal lenses in a retrospective analysis of 62 patients with KCS due to cGvHD following allo-CST.

All patients had superficial punctate keratitis refractory to standard treatments. Evaluation of patients was carried out by two ophthalmologists. cGVHD was recorded according standard criteria. Ocular surface disease index (OSDI) and Oxford score were used to evaluate ocular symptoms. The scale of "Monoyer" was used and converted into visual acuity "LOG MAR" for comparative purposes of the study.

**Results:** All patients but two agreed to hold the lenses. The 62 patients were equipped with sclero-corneal lenses. With a median follow-up of 22 months (IQR 8-43) from the treatment with lenses, all patients have experienced an improvement in their quality of life with a clear improvement of dry-eye symptoms. This quality of life is also improved by decreasing the frequency of eye-drop instillation and the attenuation of the discomfort and post-instillation visual fluctuation. At two months, all patients but one experienced improvement in OSDI score with a median of 92 points (range, 40-100) before lenses to a median of 25 points (range, 3-90). We also observed improvement or stability of visual acuity with median gain of -0.2 (range, -1 to +0.1) LOG MAR acuity and improvement or stability of the Oxford score with a median of 3 points (range, 0-5) before lenses to a median of 1 point (range, 0-4) after lenses, with a median gain of 2 points in almost all patients.

**Conclusion:** To our knowledge, this is the largest cohort of patients with KCS having sclero-corneal lenses. Treatment of KCS with sclero-corneal lenses is promising. Whenever possible, this approach should be considered in patients experiencing KCS due to cGVHD.

**Disclosure of Interest:** None declared.

#### O040

### A New Approach of Dual-SCT with Unmanipulated Haplo-Identical Graft and Unrelated Cord Blood in Patients with Hematological Disorders

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**Introduction:** Dual-SCT with T-cell depleted haplo-identical graft and cord blood (CB) has developed in recent years, which revealed a promising outcome. In China, graft manipulation is seldom performed, so we proposed an alternative dual-SCT strategy with unmanipulated haplo-identical graft co-infusing with unrelated CB unit, and published an encouraging outcome based on preliminary results<sup>[1]</sup>. Here we reported the recent updates of this prospective study.

**Material (or patients) and methods:** This is an on-going prospective study approved by local Ethics Committee. The main inclusion and exclusion criteria are: (1) definitely

diagnosed as hematological disorders; (2) with an indication of allo-SCT; (3) without an available matched related or unrelated donor. Myeloablative conditioning of either modified Bu/Cy regimen or modified TBI/Cy regimen was applied. Selection of donor, conditioning, GVHD prophylaxis and supporting care follows the principles described previously<sup>[1]</sup>. In order to identify the superiority of dual-SCT, data of patients receiving haplo-identical donor transplantation in the same time frame were analyzed as parallel control in this report.

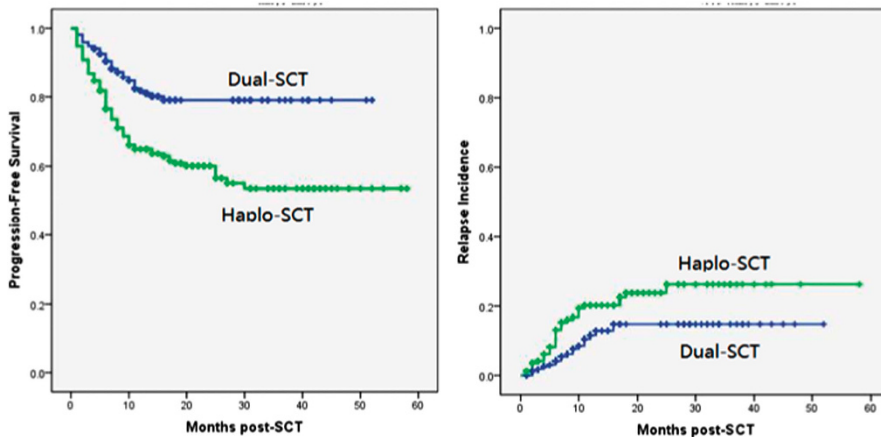
**Results:** From January 2011 through December 2014, 219 patients were recruited in this study including patients with AML (29%), ALL (38%), MDS (10%), SAA (10%) and other diseases. The median age was 26 (15-60) years old. The median counts of MNC was  $10.5 \times 10^8/\text{Kg}$  in haplo-identical graft and  $1.83 \times 10^7/\text{Kg}$  in cord blood unit, respectively. Only 4 patients achieved stable engraftment of CB unit. Univariate analysis in the study cohort suggested that lower MNC count (less than  $12 \times 10^8/\text{Kg}$ ) in haplo-identical graft was related to a significantly lower Grade II-IV aGVHD incidence ( $P=0.041$ ), lower TRM ( $P=0.008$ ) and better survival ( $P=0.007$ ). Comparing with 5/6 or 6/6 matched CB unit, 4/6 matched CB unit resulted in higher TRM with a statistical significance ( $P=0.023$ ), and lower OS with marginal significance ( $P=0.071$ ). Parallel control group contains 176 patients with roughly comparable baseline characteristics. In the comparison analysis, 2-year TRM was  $14.9 \pm 3.0\%$  versus  $29.4 \pm 5.4\%$  for dual-SCT and haplo-SCT ( $P=0.003$ ), and 2-year relapse incidence was  $14.8 \pm 3.5\%$  versus  $23.3 \pm 4.4\%$  ( $P=0.011$ ), respectively. Finally, better 2-year OS ( $81.5 \pm 3.1\%$  versus  $64.4 \pm 4.2\%$ ,  $P < 0.001$ ) and PFS ( $79.1 \pm 3.0\%$  versus  $58.9 \pm 4.1\%$ ,  $P < 0.001$ ) were observed in dual-SCT group compared to haplo-SCT (Fig 1). No difference of GVHD incidence was identified between two groups.

**Conclusion:** Our report showed promising outcomes of dual-SCT with unmanipulated haplo-identical graft and unrelated CB unit. Lower MNC count in haplo-identical graft and 5-6/6 matched CB unit were related to a better prognosis after dual-SCT. Efficacy of this approach merits further confirmation by longer follow-up, and moreover, by well designed randomized clinical trials. Besides, probable mechanisms and interactions between stem cells needs to be explored.

**References:** [1] Chen J, Wang R-X, Chen F, *et al.* Combination of a haploidentical SCT with an unrelated cord blood unit: a single-arm prospective study. *Bone Marrow Transplant*, 2014, 49: 206-211.

**Disclosure of Interest:** None declared.

[O040]



O041

**Impact of graft versus host disease prophylaxis on outcomes of unmanipulated haploidentical stem cell transplantation: post-transplant cyclophosphamide versus antithymocyte globulin based regimen**

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**Introduction:** Unmanipulated haploidentical stem cell transplantation (haplo-SCT) has been used more frequently in recent years. Severe graft versus host disease (GVHD) is a major barrier of non T-cell depleted haplo-SCT. There is no consensus on the GVHD prophylaxis. The current study aimed to compare the two most common used GVHD prophylaxis regimens (post-transplant cyclophosphamide (PT-CY) versus the anti-thymocyte globulin based in adults with acute myeloid leukemia (AML) in first or second complete remission (CR1 or CR2), reported to the EBMT registry from 2007 to 2014.

**Material (or patients) and methods:** Primary end point was leukemia free survival (LFS). Secondary end points were refined GVHD-free relapse-free survival (GRFS), grade II-IV aGVHD and chronic GVHD, relapse incidence (RI), non-relapse mortality (NRM), and overall survival (OS). Median follow up was 22 months.

**Results:** A total of 308 patients (pts) were reported from 78 EBMT transplant centers. GVHD prophylaxis was PT-CY based in 193 pts and ATG based in 115 pts. Of the 308 patients, 61% in the PT-CY and 63% in the ATG group, were transplanted in CR1 ( $P=0.71$ ). There was no difference in the conditioning regimen, being 50% MAC in both groups ( $P=0.59$ ). Pts in the two groups were comparable except that pts in PT-CY group were more likely to receive bone marrow (BM) as graft source (60.1% vs 39.9%,  $P=0.016$ ) and had shorter follow-up (18 months vs 36 months,  $P<0.001$ ).

GRFS, LFS and OS were 50.9% vs 38.9% ( $P=0.07$ ), 56% vs 47.2% ( $P=0.26$ ) and 58% vs 54.2% ( $P=0.37$ ), for patients receiving PT-CY or ATG, respectively.

According to disease status at Haplo-SCT, the 2-year LFS was 54.6% for pts in CR1 and 47.8% in CR2,  $P=0.93$ .

Grade 3-4 aGVHD, cGVHD, CI of relapse at 2-years and NRM in pts receiving PT-CY or ATG were 4.7% vs 12.5% ( $P=0.01$ ), 33.7% vs 28.3% ( $P=0.33$ ), 21.6% vs 22.3% ( $P=0.97$ ) and 22.4% vs 30.5% ( $P=0.19$ ), respectively.

Adjusted multivariate analysis showed that pts receiving ATG had a significantly lower GRFS (HR 1.45; 95% CI 1.04-2.02,  $P=0.030$ ), lower LFS (HR 1.48; 95% CI 1.03-2.12,  $P=0.034$ ), and a higher NRM (HR 1.77; 95%CI 1.09-2.86,  $P=0.02$ ) in comparison to pts receiving PT-CY. ATG based prophylaxis was associated with higher grade 3-4 aGVHD (HR 2.42; 95% CI 1.20-5.75,  $P=0.04$ ) while no association with relapse, OS and cGVHD. In addition, the number of Haplo-SCT performed per transplant center, was additional independent factor associated with GRFS (HR 0.99; 95% CI 0.97- 1.00,  $P<0.04$ ), LFS (HR 0.97; 95% CI 0.96- 0.99,  $P<0.001$ ), NRM (HR 0.96; 95%CI 0.94-0.98,  $P<0.001$ ) and cGVHD (HR 1.06; 95% CI 1.04-1.07,  $P=<0.001$ ). One hundred twenty two pts died, 62% of transplant related causes and 38% due to disease recurrence. The causes of death were not different according to the type of GVHD prophylaxis.

**Conclusion:** In conclusion, for pts with AML in CR, unmanipulated Haplo-SCT using PT-CY with no ATG as GVHD prophylaxis allowed better LFS and GRFS, lower GVHD and

lower NRM in comparison to ATG-based prophylaxis. This significant difference was observed for both BM and PBSC grafts as well as in the RIC and MAC setting.

**Disclosure of Interest:** None declared.

O042

**Donor lymphocytes depleted of alloreactive T-cells (ATIR101) improve overall survival and reduce transplant related mortality in a T-cell depleted haploidentical HSCT: Results from a Phase 2 trial in patients with AML and ALL**

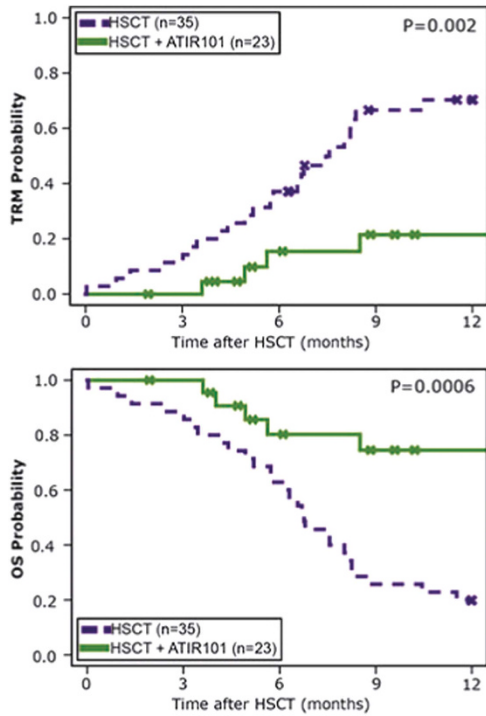
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**Introduction:** Haploidentical donors may resolve shortage of available HLA-matched donors for treatment of blood cancer with a hematopoietic stem cell transplantation (HSCT). However, to prevent graft-versus-host disease (GVHD), haploidentical HSCT requires alloreactive T-cell depletion. We developed a strategy that allows additional donor lymphocytes to be infused post-HSCT without risk of inducing severe GVHD and maintaining reactivity against viruses and leukemic cells.

**Material (or patients) and methods:** In this open-label, multicenter phase 2 study (CR-AIR-007; NCT01794299), 23 patients with median age of 41 years (range 21-64) were treated with ATIR101. Sixteen patients had AML (70%), 11 in CR1 and 5 in CR2, and seven patients had ALL (30%), 4 in CR1 and 3 in CR2/3 at time of HSCT. Patients underwent myeloablative conditioning, consisting of a) TBI (1200 cGy;  $n=11$ ) or b) melphalan (120mg/m<sup>2</sup>;  $n=12$ ), along with thiopeta (10 mg/kg), fludarabine (30 mg/m<sup>2</sup> x 5d) and ATG (2.5mg/kg x 4d). A CD34+ selected stem cell graft from a haploidentical donor was given, containing  $11 \times 10^6$  CD34+ cells/kg (range:4.7-24.4) and  $0.29 \times 10^4$  CD3+ cells/kg (range; 0-1.8). In addition, donor lymphocytes from the same donor were processed using a selective photodepletion technology, creating a donor lymphocyte infusion depleted of alloreactive T-cells (ATIR101). ATIR101 was infused at a median of 28 days (range; 28-73) post-HSCT at a fixed dose of  $2 \times 10^6$  CD3+ cells/kg, without use of post-transplant GVHD prophylaxis.

**Results:** All patients engrafted rapidly after transplantation, with neutrophil and platelet engraftment achieved at a median of 12 days (range 8-34 and 9-35 respectively). Median follow-up (as of November 23<sup>rd</sup>, 2015) is 292 days post-HSCT. A total of 19 patients were beyond 6 months post-HSCT, of which 15 were alive at that time, and 15 patients were already 12 months post-HSCT, of which 10 were alive. No patients developed grade III/IV acute GVHD after infusion of ATIR101. Two cases of grade II acute GVHD were reported with a delayed onset, starting only at day 173 and day 247 post-HSCT. No patient died within 100 days post-HSCT and there were 3 deaths as a result of TRM (all infections) within 6 months post-HSCT. When compared to a historic control group (N=35), TRM was significantly lower in patients given ATIR101 after a T-cell depleted HSCT with a 6-month TRM for HSCT + ATIR101 of 15% versus 37% for HSCT only (Figure 1a). Thus far two patients experienced a relapse within the first year, occurring at 60 and 90 days, respectively, post-HSCT. One patient died as a result of the disease relapse. The overall survival of patients given ATIR101 was significantly improved compared to a historic control group, with a 1-year survival of



75% in the HSCT+ATIR101 group and 20% in the control group (Figure 1b).

**Conclusion:** Administration of a high dose of donor lymphocytes in ATIR101 from a haploidentical donor does not cause severe GVHD without use of prophylactic immune suppression. Addition of ATIR101 to a T-cell depleted HSCT protocol significantly improves transplantation outcome, with reduced TRM and improved OS. Moreover, the low number of relapses observed thus far is most encouraging and supports the hypothesis of preservation of T-cells in ATIR101 able to recognize leukemic antigens.

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#### O043

### HLA disparities on unshared haplotype influence outcomes of unmanipulated haploidentical hematopoietic stem cell transplantation (HaploSCT): A Study from the Acute Leukemia Working Party of European Society for Blood and Marrow Transplantation (EBMT)

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**Introduction:** HaploSCT is a valid option for high risk acute leukemia (AL) patients (pts) who lack a matched related or unrelated donor. We aimed to evaluate if outcomes after unmanipulated HaploSCT could be shaped by number of HLA mismatches (HLAm) or mismatches at specific HLA loci on unshared haplotype between pts and donors, possibly refining current criteria for haplo-donor selection.

**Material (or patients) and methods:** We included adult pts with *de novo* AML ( $n=403$ ) and ALL ( $n=156$ ) reported to EBMT registry who underwent an HaploSCT as their first allograft in 2007-2014. We selected 559 donor-recipient pairs with molecular HLA-A, -B, -C and -DRB1 typing at low (66%) or high (34%) resolution level. HLAm were defined at antigen level.

**Results:** Median follow-up for survivors was 19 months; median pts age at transplant was 44 (18-78) years (y); median donors age 38 (12-74) y. More than half of pts were in complete remission (36% CR1, 27% CR $\geq$ 2), 37% in active disease. Most transplants were performed using a reduced intensity conditioning regimen (55%) with busulfan- and fludarabine-based protocols being the most frequently used. Source of stem cells was peripheral blood for 60% of pts, bone marrow for 40%. In vivo T cell depletion with ATG was used in 35% of cases and 54% of pts received post-transplant Cyclophosphamide (PTCy) as backbone of GvHD prophylaxis, mostly associated with calcineurin inhibitors and mycophenolate mofetil.

Overall, 55% of pairs had 4 HLAm on unshared haplotype, 30% had 3 HLAm, 12% had 2 HLAm, 3% had 1 HLAm. Among pairs with less than 4 HLAm, 17% were matched at locus A, 11% at locus B, 18% at locus C and 17% at locus DRB1. In multivariate analysis including potential confounding factors (center effect, pts and donor age and sex, AL type, disease status, conditioning regimen intensity, stem cells source, *in vivo* T-cell depletion, PTCy), increasing number of HLAm was not an independent risk factor for acute GVHD

(HR 0.8, p 0.3), chronic GVHD (HR 1.2, p 0.5), non-relapse mortality (NRM) (HR 1.2, p 0.4), relapse (HR 1, p 0.9), leukemia-free survival (HR 1.1, p 0.6) and overall survival (HR 1.2, p 0.3). When the impact of match at specific loci was examined, mismatch at DRB1 locus was associated with higher cumulative incidence of grade 2-4 aGVHD at 100 days ( $34 \pm 5\%$  in mismatched pairs,  $21 \pm 8\%$  in matched pairs, p 0.01). Moreover, mismatch at B locus was associated with higher 2y NRM ( $30 \pm 11\%$  in mismatched pairs,  $16 \pm 8\%$  in matched pairs, p 0.03), mainly due by a tendency to higher rates of infections in mismatched compared to matched pairs ( $22 \pm 2\%$  and  $13 \pm 7\%$  at 2y, respectively, p 0.08). After adjusting for covariates in multivariate analysis, mismatch at DRB1 was an independent risk factor for grade 2-4 aGVHD (HR 1.8, p 0.01) and mismatch at B locus was an independent risk factor for NRM (HR 2, p 0.04).

**Conclusion:** In our series of 559 AL pts, number of HLA mismatch on unshared haplotype did not influence unmanipulated HaploSCT outcomes. However, mismatches at DRB1 locus and at B locus were independent risk factors for grade 2-4 aGVHD and NRM, respectively. Further studies will be needed to reveal if selection of haplo-donors based on optimal HLA matching could be useful to reduce some of the excess of morbidity associated with unmanipulated HaploSCT.

**Disclosure of Interest:** None declared.

#### O044

##### Reconstitution of T cell subsets after haploidentical hematopoietic cell transplantation using alpha beta T cell-depleted graft and the clinical implication of $\gamma\delta$ T cells

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**Introduction:**  $\alpha\beta$  T cell-depleted haploidentical hematopoietic cell transplantation (HHCT) allows transfer of not only high numbers of CD34+ cells and "innate-like" immune cells such as  $\gamma\delta$  T cells, which can exert protective effects against leukemia cell growth and life-threatening infections. This study investigated reconstitution of T cell subsets early after  $\alpha\beta$  T lymphocyte-depleted HHCT and the clinical implications of peripheral  $\gamma\delta$  T cells in pediatric patients.

**Material (or patients) and methods:** We analyzed forty-one pediatric patients who received HHCTs using  $\alpha\beta$  T cell-depleted grafts between August 2012 and July 2015. Reconstitution of T lymphocyte subsets was monitored in peripheral blood by flow cytometry at day 14, 30, 60, 90, and 180 ( $\pm 10$  days). The median age was 12.9 years. Diagnosis included acute leukemia ( $n=20$ ), severe aplastic anemia ( $n=6$ ), and others ( $n=15$ ).

**Results:** The number of CD3+ T cells and CD8+ T cells recovered rapidly and reached donor levels at days 180 and 60, respectively. However, CD4+ T cell recovery was delayed and did not reach donor levels by day 180. Approximately 42% of patients showed  $> 100$  CD4+ T cells/ $\mu$ l at day 60, and 76% of patients showed  $> 100$  CD4+ T cells/ $\mu$ l at day 180. Delayed reconstitution of CD4+ T cells and the rapid CD8+ T cell immune reconstitution led to an inverse CD4/CD8 ratio consistently during the first 6 months after HHCT. At day 14 after HHCT,  $\gamma\delta$  T lymphocytes predominantly consisted of the CD3+ T cells, with a median of 73.0%. The  $\gamma\delta$  T cell population then gradually decreased, while the percentage of  $\alpha\beta$  T cells gradually increased. On day 14 after HHCT, analyses of  $\gamma\delta$  T cell subsets showed that 19.2% were naïve, 31.7% were central memory, and 2.1% were effector memory. The percentage of naïve  $\gamma\delta$  T lymphocytes gradually increased between days 14 and 60 after HHCT, with statistical significance between days 30 and 60 ( $P < 0.01$ ). Patients with a low percentage ( $\leq 21\%$ ) of

$\gamma\delta$  T cells at day 30 had significantly higher incidence of cytomegalovirus (CMV) reactivation compared to patients with a high percentage ( $> 70\%$ ) of  $\gamma\delta$  T cells ( $100\%$  vs.  $25.0 \pm 21.7\%$ ,  $P=0.04$ ), suggesting protective role of  $\gamma\delta$  T cells on CMV reactivation. Grade II-IV acute GVHD occurred in 11 patients at a median 17 days.  $\gamma\delta$  T cells at day 14 were not associated with acute GVHD. However, patients with a high percentage of  $\gamma\delta$  T cells at day 30 showed no grade II-IV acute GVHD, while patients with low/average percentage of  $\gamma\delta$  T cells showed higher incidence of grade II-IV acute GVHD ( $40.9 \pm 10.0\%$ ,  $P=0.05$ ). Data is limited to determine if  $\gamma\delta$  T cells at day 30 represent the immunologic status of acute GVHD itself or consequences of immune suppressants for acute GVHD in  $\alpha\beta$  T cell-depleted HHCT. At day 30, percentages of  $\gamma\delta$  T cells in patients who relapsed were lower compared with patients who did not relapse with borderline statistical significance (median 33.3% vs. 51.6%,  $P=0.05$ ).

**Conclusion:** This study established detailed data of reconstitution of T cell subsets after  $\alpha\beta$  T cell-depleted HHCT, and provided a platform for further researches regarding immune reconstitution after HHCT. Data suggests that early recovery of  $\gamma\delta$  T cells decreases the risk of CMV reactivation. Further studies are needed to clarify the role of  $\gamma\delta$  T cells in GVHD and their anti-leukemic effects in properly designed large clinical trials.

**Disclosure of Interest:** None declared.

#### O045

##### Sequential therapy in the setting of HLA-haploidentical transplantation utilizing T-cell-replete grafts and post-transplantation high-dose cyclophosphamide in the treatment of relapsed and refractory acute myeloid leukemia and myelodysplastic syndrome

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**Introduction:** Due to a safe toxicity profile HLA-haploidentical transplantation (haplo-HSCT) using T-cell-replete (TCR) grafts and high-dose cyclophosphamide post-transplantation (PTCY) has found increasing acceptance. However, in patients with high-risk and advanced disease outcome was limited due to a high relapse incidence, in particular when the underlying disease was of myeloid origin. In HLA-matched transplantation it has been shown that sequential therapy comprising cytoreductive chemotherapy and reduced intensity conditioning (RIC) can provide sufficient disease control and favourable outcome in patients with high-risk and refractory AML and MDS (Schmid C JCO 2005).

**Material (or patients) and methods:** To evaluate the feasibility and outcome of sequential therapy in the context of haplo-HSCT utilizing TCR grafts and PTCY in patients with relapsed and refractory AML and MDS, we retrospectively analyzed the course of 35 patients (AML  $n=25$ ; sAML  $n=7$ , MDS  $n=3$ ; median age: 62 years; male  $n=17$ ) transplanted between 2009 and 2015. All patients presented with active disease at time of transplant (primary refractory  $n=6$ ; relapsed  $n=7$ ; relapsed and refractory  $n=16$ ; upfront  $n=5$ ). All patients received sequential therapy prior to haplo-HSCT combining cytoreductive chemotherapy (FLAMSA  $n=19$ ; clofarabine  $n=14$ ; others  $n=2$ ) and RIC. Conditioning was drug-based in 23 patients receiving fludarabine, cyclophosphamide (CY) and melphalan (110 mg/m<sup>2</sup>) while it was TBI-based (4 Gy) in the others ( $n=12$ ). Post-grafting immunosuppression was high-dose CY given on day +3 and +4, tacrolimus and mycophenolate mofetil (both started on day +5).

Unstimulated bone marrow was the graft source in 17 patients.

**Results:** No graft rejection was observed. Neutrophil/platelet engraftment was achieved in 30/28 of 30 evaluable patients at a median of 16 and 33 days, respectively. Acute GvHD grade II-IV occurred in 12 patients (34 %) while it was severe in only 2 (6 %). Chronic GvHD was observed in 7 patients (20 %), and was moderate in 3 (9 %) and severe in 2 patients (6 %). No patient developed VOD. Kidney failure requiring hemodialysis occurred in 4 patients. CMV reactivation was observed in 14 of 26 patients at risk (54 %), while only one patient developed CMV colitis; no PTLD was observed. Invasive fungal infection was diagnosed 8 patients. One-year CI of NRM was 21 % (95% CI 9-36). CR and fully donor chimerism was achieved in 27 patients at day +30. Eleven patients relapsed; one and two-year CI of relapse was 33 % (95 % CI 17-49), both. After a median follow up of 28 months (range: 4.6-64), one-year and two-year overall survival (OS) was 51 % (95 % CI 34-67) and 44 % (95 % CI 27-61), respectively. One and two-year relapse-free survival (RFS) was 47% (29-62), both.

**Conclusion:** Sequential therapy in the setting of TCR haplo-HSCT using RIC and PTCy is well tolerated with low rates of GvHD and acceptable NRM in patients with advanced AML and MDS, while providing an effective anti-leukemic activity in relapsed and refractory disease. Thus, we suggest that by utilizing sequential therapy in TCR/PTCY haplo-HSCT donor availability can be expanded in patients with advanced AML/MDS who lack a conventional donor or need promptly access to a donor due to aggressive disease.

**Disclosure of Interest:** None declared.

#### O046

##### **Refined graft-versus-host disease-free/relapse-free survival - a novel outcome endpoint for haploidentical transplantation in adults with acute leukemia: on behalf of ALWP-EBMT**

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**Introduction:** Allogeneic stem cell transplant (HSCT) from haploidentical (haplo) donor is increasingly used for patients (pts) with acute leukemia (AL). Both graft manipulation with ex vivo T-cell depletion (TCD) and *in vivo* TCD mainly with ATG or recently by using post-transplant cyclophosphamide (PTCy) are successfully performed. The novel composite endpoint of graft-versus-host disease (GVHD)-free, relapse-free survival (GRFS) represents an important measure of clinical benefit of HSCT without ongoing morbidity.

**Material (or patients) and methods:** We analyzed the refined GRFS in 730 adults with de novo AL, who received a haplo either with ex vivo TCD ( $n=188$ ), or T-cell repleted with *in vivo* TCD (ATG-TCR) ( $n=180$ ) or non ATG-based (non-ATG-TCR) ( $n=362$ ) between 2007 and 2014 and reported to EBMT.

**Results:** Median follow-up was 20 months. The majority of pts had acute myeloid leukemia (AML) (TCD 72%, ATG-TCR 74% and non-ATG-TCR 70%,  $P=0.5$ ). Disease status at haplo was first complete remission (CR1) in 60% of pts. Stem cell source was peripheral blood cells (PB) for 95% of the pts with TCD, 55% in the ATG-TCR and 42% in the non-ATG-TCR ( $P<0.001$ ). Conditioning regimen was different among the 3 groups: being MAC in 70% of the TCD vs 54% in the ATG-TCR vs 59% in the non-ATG-TCR ( $P=0.003$ ). Among pts in the non-ATG-TCR, 79% received PTCy. There was no difference in the cumulative incidence (CI) of neutrophil engraftment (96%, 96% and 95%,  $P=0.76$ ) and grade II-IV acute GVHD (23%, 29% and 28%,  $P=0.27$ ) between the 3 groups. cGVHD was lower in pts receiving a TCD graft (19%, 32% and 35%,  $P<0.001$ ). CI of relapse was 18%, 23% and 26% ( $P=0.11$ ) in TCD, ATG-TCR and non-ATG-TCR. CI of non-relapse mortality (NRM) was significantly higher in the TCD versus the T replete groups (50% vs 36% vs 20%,  $P<0.001$ ). Similarly, TCD haplo-SCT were associated with lower leukemia free survival (LFS) at 2-year in comparison to ATG-TCR and non-ATG-TCR (32% vs 41% vs 53%,  $P<0.001$ ) and lower rGRFS (28%, 31% and 45%,  $P<0.001$ ). In subgroup analysis 2-year LFS was higher in the non-ATG-TCR for AML,  $P<0.001$  and acute lymphoblastic leukemia (ALL),  $P=0.03$ . For pts in CR1, 2-year LFS was 40% vs 46% vs 56% ( $P=0.13$ ) while in CR2 it was 20% vs 33% vs 50%, ( $P<0.001$ ) in TCD, ATG-TCR and non-ATG-TCR, respectively. In multivariate analysis, relapse incidence (RI) was higher in pts with ALL (HR 1.49, 95%CI 1.04-2.13,  $P=0.03$ ) and in case of CMV neg donors (HR 1.5, 95% CI 1.04-2.96,  $P=0.03$ ). Disease status (CR1 vs CR2: HR 0.64, 95%CI 0.48-0.86,  $P=0.003$ ), use of unmanipulated graft (ATG-TCR: HR 0.71, 95%CI 0.49-1.04,  $P=0.07$  and non-ATG-TCR: HR 0.38, 95%CI 0.26-0.55,  $P<0.001$ ), and RIC (HR 0.70, 95%CI 0.52-0.95,  $P=0.02$ ) were independently associated with lower NRM. Diagnosis of AML (HR 0.78, 95%CI 0.63-0.98,  $P=0.03$ ), CR1 (HR 0.48, 95%CI 0.54-0.86,  $P<0.001$ ) and non-ATG-TCR (HR 0.60, 95%CI 0.46-0.80,  $P<0.001$ ) were associated with better LFS. Use of bone marrow (BM) (HR 0.76; 95%CI 0.61-0.96,  $P=0.02$ ) and non-ATG-TCR graft (HR 0.73, 95%CI 0.56-0.95,  $P=0.02$ ) significantly improved rGRFS.

**Conclusion:** Unmanipulated haplo without *in vivo* TCD is associated with higher rGRFS, LFS and low NRM, when compared with ex vivo TCD or *in vivo* ATG based TCD graft. rGRFS is also significantly higher with BM in comparison to PB grafts.

**Disclosure of Interest:** None declared.

#### O047

##### **cotransplantation of bone marrow-derived mesenchymal stem cells in haploidentical hematopoietic stem-cell transplantation in patients with severe aplastic anemia: multicenter phase II trial results**

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**Introduction:** Haploidentical hematopoietic stem-cell transplantation (haplo-HSCT) for severe aplastic anemia (SAA) is mainly associated with graft failure and graft-versus-host disease (GVHD). Mesenchymal stem cells (MSCs) have been shown to support normal hematopoiesis *in vivo* and to display potent immunosuppressive effects to prevent or treat GVHD after HSCT.

**Material (or patients) and methods:** In a multicenter phase II trial, 44 patients with SAA were included, who were new diagnosed or failed to response to previous

immunosuppressive therapy and were transfused frequently. We developed an approach with cotransplantation the culture-expanded third-party related-donor-derived bone marrow (BM)-MSCs in patients undergoing alternative donor transplantation without T-cell-depletion. 3.6 (range 3.2 to 4.1) × 10<sup>6</sup>/kg BM-MSCs were transfused to patients by venous for 2 times, 6 hours before the infusion of hematopoietic stem cells at day 01 and at day +14 after transplantation. The conditioning regimens included busulfan (BU), cyclophosphamide (CY) and thymoglobulin (ATG). The recipients received cyclosporin A (CsA), mycophenolate mofetil (MMF) and short-term methotrexate (MTX) for GVHD prophylaxis. Three of 44 patients died before stem cell engraftment and were excluded.

**Results:** Only one patient didn't achieve hematopoietic reconstitution among the 41 evaluable patients. The median time for myeloid engraftment was 12 days (range 8–21 days) and for platelets engraftment was 19 days (range 8–154 days). The 41 evaluable patients achieved sustained full donor chimerism without any adverse BM-MSC infusion-related events. The incidence was 29.3% for grade II–IV acute GVHD and 14.6% for chronic GVHD. The overall survival (OS) was 77.3% with a median 12-month (0.9–30.8) follow-up for surviving patients.

**Conclusion:** These data suggest that cotransplantation of bone marrow derived mesenchymal stem cells from related donors could reduce the risk of graft failure and severe GVHD in alternative donor transplantation for SAA.

**Disclosure of Interest:** None declared.

#### O048

##### Antimicrobial resistance of Gram-negative bacteria isolated from blood in HSCT recipients: a multinational prospective study on behalf of the EBMT-IDWP

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**Introduction:** We report on resistance rate in Gram-negative rods (GNR) in HSCT patients, risk factors for resistance and mortality in bacteremia episodes caused by resistant vs. sensitive GNR.

**Material (or patients) and methods:** Data on all episodes of GNR bacteremia since the initiating of conditioning and during 6 months after the HSCT were collected prospectively by a special form, containing information on pathogen, antimicrobial susceptibility, the risk factors, treatment and outcome. Background patient demographic and clinical data were

obtained using MED A form of ProMise. Four patterns of resistance were assessed: to quinolones, non-carbapenem beta-lactams (at least one of ceftazidime, cefepime or piperacillin-tazobactam); carbapenems (at least one of meropenem/imipenem/doripenem); multidrug resistance (MDR, at least 1 agent in ≥ 3 any antimicrobial categories)<sup>1</sup>.

**Results:** 444 patients (median age at SCT 51 years, range 0.5-73; 267 males) developed 485 episodes of GN bacteremia in 58 HSCT centers from 23 countries. 181 patients underwent autologous, 263 allogeneic HSCT (65% of them received myeloablative conditioning). The stem cells sources were: 343 peripheral blood, 72 bone marrow, 10 both, 18 cord blood, 1 missing. Neutropenia < 500 and < 100 cells/mm<sup>3</sup> was present in 77% and 72% of episodes, respectively; breakthrough bacteremia occurred in 61%; GVHD (43 acute and 8 chronic) in 17% of allo-HSCT episodes.

In 93% episodes, one GNR was isolated from blood, in 9% of them a GNR was isolated together with Gram-positive, anaerobe or *Candida* spp.; in 7% of episodes ≥ 2 GNR were isolated from blood.

Total 519 GNR were isolated; 73% *Enterobacteriaceae*, 24% nonfermentatives, 3% others. Resistance to fluoroquinolones was recorded in 53%, to non-carbapenem beta-lactam in 47%, to carbapenems in 16%, MDR in 54% of isolates.

30-days mortality was 15% (61/401); in 84 episodes this information yet unavailable. Mortality according to resistance pattern was as follows: in those resistant vs. sensitive to non-carbapenem beta-lactam: 21% vs. 9%, *P* = 0.002; in carbapenem-resistant vs. sensitive: 40% vs. 10%, *P* < 0.001; in MDR vs. non-MDR: 21% vs. 8%, *P* = *P* < 0.001.

Multivariate analysis of risk factors for resistance appears in the Table.

Table.

Risk factor	Resistance to:			
	Non-carbapenem beta-lactam	Carbapenems	MDR	Quinolones
	OR (95%CI), P value			
Urinary catheter at time of bacteremia	4.9 (1.3-17.8) 0.017	Non-significant (NS)	NS	NS
Breakthrough bacteremia	2.6 (1.5-4.4) < 0.001	3.4 (1.5-8.0) 0.004	2.4 (1.5-3.8) (1.5-3.8)	6.8 (4.0-11.4) < 0.001
Previous (last 3 months) antibiotic treatment	2.0 (1.2-3.3) 0.009	NS	2.9 (1.8-4.6) < 0.001	NS
Neutropenia (< 500 cells/mm <sup>3</sup> )	NS	NS	2.0 (1.2-3.5) 0.009	1.9 (1.1-3.5) 0.029
Steroid treatment during the last month	NS	NS	1.7 (1.0-2.8) 0.038	NS
Previous bacteremia	NS	2.0 (1.0-4.0) 0.045	NS	NS

**Conclusion:** The emergence of antibiotic resistance represents a major obstacle to successful outcome of GN bacteremia after HSCT, especially in case of carbapenem-resistance. The high rate of fluoroquinolone resistance questions the value of prophylaxis. Main risk factors for resistance are breakthrough bacteremia (for any antibiotic resistance), having urinary catheter (beta-lactam only), previous bacteremia (carbapenem only), previous antibiotic treatment (beta-lactam and MDR), neutropenia (quinolones and MDR) and steroid treatment (MDR only).

Reference: <sup>1</sup>Magiorakos AP *et al.* Clin Microbiol Infect 18(3): 268-281,2012.

**Disclosure of Interest:** None declared.

O049

**Comparison of fungal prophylaxis with alternative dosing strategies in pediatric patients undergoing hematopoietic stem cell transplantation: a retrospective review**

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**Introduction:** Pediatric patients undergoing hematopoietic stem cell transplant (HSCT) are at increased risk for opportunistic infections, including invasive fungal infections (IFI). There is no gold standard agent for prophylaxis in these patients. At Cincinnati Children's Hospital Medical Center (CCHMC), three alternative dosing strategies for antifungal prophylaxis are utilized in the allogeneic HSCT population: genotype-directed voriconazole, alternate-day micafungin and once-weekly liposomal amphotericinB (L-AmB). There is limited data supporting the efficacy of these regimens and no studies comparing superiority. The aim of our study was to assess the safety and efficacy of these regimens in preventing IFIs when used in pediatric allogeneic HSCT patients.

**Material (or patients) and methods:** A retrospective chart review was conducted to evaluate patients less than or equal to 18 years of age who received an allogeneic HSCT between January 2010 and July 2015 at CCHMC. The primary aim was to compare efficacy of the three primary prophylactic strategies in pediatric allogeneic HSCT patients. Secondary aims included: safety comparison and assessment of pharmacoeconomic implications.

**Results:** Between 2010-2015 CCHMC performed 396 allogeneic transplants in 374 patients. A total of 244 patients met inclusion criteria. Seventy-eight patients (32%) received weekly L-AmB, 98 patients (40%) received every other day micafungin and 68 patients (28%) received genotype-directed voriconazole. Six patients (2.5%) included in the study developed an invasive fungal infection within the first 100 days post stem cell infusion. Three occurred in the micafungin group (*Candida parapsilosis*, 3 separate patients), two in the voriconazole group (*Saccharomyces cerevisiae* and *Candida glabrata*) and one in the L-AmB group (*Candida glabrata*). A total of eighteen (7.4%) suspected infections occurred defined as therapy alteration from prophylactic to treatment for a minimum of 10 days and documented intention to treat. Thirteen (72.2%) of the suspected infections occurred in the micafungin group. However, micafungin demonstrated a superior safety profile as only 2 of the documented 103 adverse events resulting in drug discontinuation occurred in this group compared to 43 in L-AmB group and 58 in the voriconazole group.

**Conclusion:** This is the first study comparing these three antifungal regimens with alternative dosing strategies in the pediatric HSCT population. The overall incidence of breakthrough fungal infections was 9.8% in the first 100 days post-transplant. This is similar to the rate of breakthrough fungal infections published in the pediatric HSCT literature. Rates of proven breakthrough infection were similar for Micafungin and voriconazole groups (3.06% and 2.94% respectively). Weekly L-AmB regimen had the lowest rate of proven breakthrough infections at 1.2%. Patients in the micafungin group had more suspected infections, leading to change in therapy. However, in terms of safety profile, alternate day micafungin had the lowest incidence of adverse events. Sub analyses for patients in the suspected fungal infection group is ongoing to further delineate which patients had active GVHD and were on increased immune suppression. These data along with the pharmacoeconomic results will add more insight into risk and benefit balance between each of these regimens.

**Disclosure of Interest:** None declared.

O050

**Longitudinal microbiome profile associates with the risk of major transplant-related complications in allogeneic HSCT recipients**

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**Introduction:** Allogeneic HSCT (allo-HSCT) is the only cure for several patients (pts) with hematological malignancies. Recent advances in therapies have significantly reduced treatment-related mortality; nevertheless infections and graft-versus-host disease (GvHD) still represent major complications. Recent studies indicate that pts undergo dramatic alterations of intestinal microbiota during allo-HSCT, potentially affecting the outcome.

**Material (or patients) and methods:** Between October 2014 and June 2015, we have conducted a prospective observational study to examine the intestinal microbiota by NGS techniques in 44 consecutive adult pts (median age 52), who received allo-HSCT for high-risk hematological malignancies (61% acute leukemia). Stem cell donors were family haploidentical ( $n=18$ ), HLA identical sibling ( $n=9$ ), unrelated volunteer ( $n=14$ ), cord blood ( $n=3$ ). Stem cell source was mainly T-cell replete PBSCs (86%). Fecal specimens have been collected before conditioning (T0), during aplasia (T10) and after engraftment (T30). The fecal microbiome have been analyzed using the 454 GS Junior System, and QIIME software. We defined a threshold of normality for the three main phyla on the basis of the control (Proteobacteria: 5%, Firmicutes: 70%, Bacteroidetes: 60%).

**Results:** We observed an important difference in relative percentages of phyla populating the gut between our pts and control. Our pts showed a progressive reduction of the intestinal microbial diversity (alpha diversity) during the transplant process, with noteworthy changes in the resilient bacterial populations. A great variation was observed, particularly between T0 and T30, where we found a significant decrease in alpha diversity ( $p < 0.01$ ).

Pts ( $n=6$ ) who developed sepsis by gram-negative multi-drug-resistant (GN-MDR) bacteria show a trend towards an increase of Proteobacteria phylum ( $P=0.06$  and relative risk=4.42 at T0;  $P=0.04$  at T10), and a decrease in Firmicutes phylum ( $P=0.04$  at T10). A more deep analysis within the Proteobacteria phylum of these pts identified a significant increase of the Enterobacteriaceae family ( $P=0.05$  at T0;  $P=0.03$  at T10). These changes in gut composition preceded the development of sepsis and the positivity for GN-MDR bacteria at routine rectal swab in many cases (82%). No statistical difference was found by alpha diversity analysis. We identified some association between the relative increase of a specific phylum and clinical variables. Acute leukemias, previous allo-HSCT and prior antimicrobial therapy represented strong risk factors for developing colonization by Proteobacteria (exceeding 5%). Interestingly, pts who developed a peri-engraftment syndrome and acute GvHD displayed a different distribution in the microbial population. The presence of Firmicutes at T0 was significantly higher in pts who experienced a peri-engraftment syndrome ( $P=0.01$ ); this observation was confirmed at T10 for pts who developed peri-engraftment syndrome and/or acute GVHD within 100 days after HSCT ( $P=0.05$ ).

**Conclusion:** Longitudinal study of microbiome profile evaluating the diversity and changes within the bacterial populations inhabiting the gut, could be applied to early identification of pts at risk for major transplant-related complications as sepsis by GN-MDR or acute GVHD, allowing pre-emptive or therapeutical strategies, and potentially improving the outcome of allo-HSCT.

**Disclosure of Interest:** None declared.



O051

**Comparative analysis of the *in vitro* immune response against *Aspergillus fumigatus* conidia in healthy blood donors versus patients after allogeneic haematopoietic stem cell transplantation**

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**Introduction:** The opportunistic human pathogen *Aspergillus fumigatus* is the most important cause of fatal fungal infections in immunocompromised hosts, such as patients after allogeneic haematopoietic stem cell transplantation (alloHSCT). In general, the infection is established by fungal conidia which are ubiquitously present in the environment. To date, a reliable diagnosis is difficult and treatment options are limited resulting in high mortality rates of infected patients while pathogenetic mechanisms are still incompletely understood. Phagocytes, i.e. monocytes and neutrophil granulocytes, are in the first line of defense against invading fungi. We sought to elucidate potentially different responses of human phagocytes towards *A. fumigatus* from healthy individuals and patients after alloHSCT. This should foster an improved understanding of the mechanism of invasive fungal infection.

**Material (or patients) and methods:** Leukocytes were prepared from blood samples from immunosuppressed patients after allogeneic haematopoietic stem cell transplantation or from buffy coats of healthy blood donors. Cells were co-incubated with resting or pre-swollen FITC-labelled *A. fumigatus* conidia for 0.5, 2 and 4 hours. Leukocytes without conidia served as control. Afterwards, cells were analysed by flow cytometry for phagocytosis as well as monocyte and neutrophil antigen marker expression.

**Results:** Up to 63% of leucocytes from healthy donors performed phagocytosis of *A.fumigatus* conidia. This maximum was achieved by neutrophils after 2 hours and by monocytes after 4 hours of co-incubation. Preliminary analysis of patient-derived cells revealed no significant difference in monocyte behaviour but phagocytosis by neutrophils was massively impaired (35% or lower) at all time points. The known upregulation of neutrophil-specific CD66b upon cell activation was observed in both healthy and immunosuppressed subjects. However, in healthy individuals, CD11b on neutrophils was upregulated after 0.5 hours of co-incubation compared to conidia-free controls and returned to basal levels within 4 hours. In contrast, CD11b expression in patient-derived neutrophils remained at elevated levels after an initial increase. These effects could not be observed in monocytes. Instead, upon co-incubation monocytes and neutrophils of both healthy and immunosuppressed origin showed a massive down-regulation of CD33 and monocytes also of CD274.

**Conclusion:** Compared to healthy individuals, we assume a delayed response of neutrophils against *A. fumigatus* conidia in patients after allogeneic stem cell transplantation due to a decreased phagocytosis rate as well as the lack of upregulated CD11b returning to a basal level. These findings might lead to a better understanding of the pathogenesis of invasive fungal infections in patients after allogeneic stem cell transplantation and could improve prophylaxis and treatment of these diseases.

**References:** Montesinos, P., R. Rodriguez-Veiga, et al. (2015). "Incidence and risk factors of post-engraftment invasive fungal disease in adult allogeneic hematopoietic stem cell transplant recipients receiving oral azoles prophylaxis." *Bone Marrow Transplant* 50(11): 1465-1472.

Hasenberg, M., J. Behnsen, et al. (2011). "Phagocyte responses towards *Aspergillus fumigatus*." *Int J Med Microbiol* 301(5): 436-444.

**Disclosure of Interest:** None declared.

O052

**Peptide vaccination against cytomegalovirus (CMV) elicits both cellular and humoral immune responses clearing the virus load after allogeneic stem cell transplantation from a CMV seronegative donor**

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**Introduction:** Patients receiving an allogeneic stem cell graft from a cytomegalovirus (CMV) seronegative donor are particularly prone to CMV reactivation with a high risk of disease and mortality. The CMV phosphoprotein 65-derived peptide NLVPMVAVT is highly immunogenic. Therefore we performed a clinical phase I peptide vaccination trial with this peptide in a water-in-oil emulsion (Montanide™) plus administration of granulocyte-macrophage colony stimulating factor (GM-CSF) (EudraCT number: 2010-018884-40).

**Material (or patients) and methods:** Ten patients after allogeneic stem cell transplantation received four vaccines s.c. at a biweekly interval. Patients were monitored for the clinical course and CMVpp65 antigenemia. CMV-specific CD8<sup>+</sup> and gamma-delta T cells were analyzed by multi-color flow cytometry. Neutralizing anti-CMV antibody assays were established and correlated to clinical parameters.

**Results:** Peptide vaccination was well tolerated and no drug-related serious adverse events were detected. Seven of nine patients with CMVpp65 antigenemia cleared the CMV after four vaccinations and are still free from viremia until now. Two patients with CMV reactivation showed no clinical response, i.e. persisting CMV antigenemia. One patient received prophylactic vaccination and did not develop viremia.

An increase in frequency of both CMV specific CD8<sup>+</sup> T cells Vdelta2-negative gamma-delta T cells was detected in four patients by factor 6 and 7, respectively. Also titers of neutralizing antibodies increased in four patients up to tenfold over the time of vaccination. Humoral and cellular immune responses correlated with clearance of the CMV load in the patients.

**Conclusion:** In summary, administration of CMVpp65 peptide vaccination for patients after allogeneic stem cell transplantation at high risk for CMV reactivation was safe, well tolerated and clinically encouraging. Further studies with larger patient cohorts are planned.

**Disclosure of Interest:** None declared.

O053

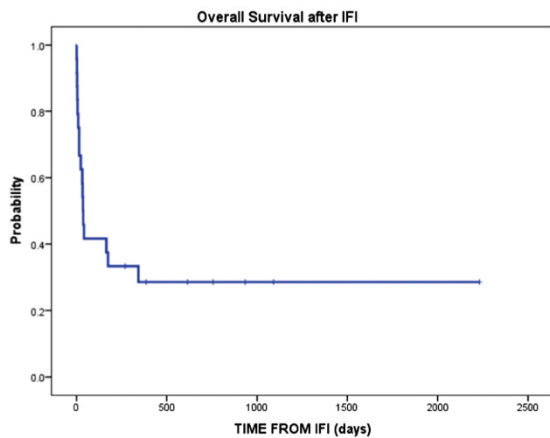
**Sequential systematic anti-mold prophylaxis with micafungin and voriconazole results in very low incidence of invasive mold infections in patients undergoing allogeneic hematopoietic cell transplantation**

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**Introduction:** Recipients of cord blood (CBT), T-cell depleted (TCD) and mismatched unrelated donors (URD) allografts are

Figure. Overall survival after IFI diagnosis (n=22).



at high risk for invasive mold infections (IMI). The impact of mold-active prophylaxis on the incidence and outcomes of mold infections is not well defined.

**Material (or patients) and methods:** We conducted a retrospective review of 988 consecutive adults who underwent allogeneic hematopoietic cell transplantation (alloHCT) in our center from 2008 through 2014. Standard prophylaxis consisted of micafungin 150mg IV daily from admission to day (D) +7 post-HCT followed by voriconazole through at least D+100. Prophylaxis was continued beyond D+100 in patients with GVHD or use of corticosteroids. Patients with intolerance or contraindication to voriconazole received posaconazole or micafungin. Patients with IMI were identified through microbiology and pathology records. Cases meeting criteria for proven or probable according to EORTC-MSG criteria are included in the analysis.

**Results:** Median age at HCT was 54 years (range 18-75). The most common diagnoses were acute myeloid leukemia ( $n=351$ , 36%) and lymphoid malignancies ( $n=248$ , 25%). Matched-related and URD were used in 686 (69%) patients, mismatched URD and CBT in 142 (14%) and 154 (16%) patients, respectively. *Ex-vivo* TCD was used in 485 (49%) patients. Sixty-four percent of patients received myeloablative conditioning. Twenty-two patients (2.2%) developed IMI at a median of 229 days (range 7-1327) after HCT. Nineteen (86%) cases were probable and 3 (14%) proven. Microbiological diagnosis was established in 9 cases (Aspergillus=4, Mucor=2, Absidia=1, Rhizomucor=1 and concomitant Rhizopus + Aspergillus=1). Thirteen (59%) patients were diagnosed with probable IMI by positive Beta-D-Glucan assay, compatible radiological findings and host factors. Pneumonia was the most presentation ( $n=19$ , 86%). Thirteen (59%) patients had received systemic corticosteroids within 30 days prior to diagnosis, 11 for GVHD. Ten of the 22 pts (45%) were not receiving the planned anti-mold prophylaxis at the time of IMI, due to azole-related toxicity ( $n=7$ ), drug allergy ( $n=1$ ), patient preference ( $n=1$ ) and unknown reason ( $n=1$ ). Three patients were off prophylaxis as they were beyond day +100 and had no additional risk factors. No risk factors for development of IMI were identified, due to low number of events. Subgroup analysis identified HCT populations with very low risk of IMI including AML in CR1 (1/248, 0.4%), allo-HCT from HLA identical sibling donors (4/323, 1.2%) and myeloproliferative neoplasms (0/58), and others with higher risk such as CBT (4/154, 2.6%) and mismatched URD (6/142, 4.2%). In the TCD-PBSC setting, 11 of 485 patients (2.3%) developed an IMI. The 12 weeks and 1 year overall survival after IMI was 42% (95%CI 32-52) and 29% (95%CI 19-38), respectively (Figure 1). All 5 patients with Mucorales died at a median of 23 days (1-616) of diagnosis (4/5 related to IMI).

**Conclusion:** 1) In this large and heterogeneous cohort including patients at high risk for mold infection who received

mold active prophylaxis, we observed a low incidence (2.2%) of IMI. 2) IMI associated mortality remained high, highlighting the need for improved diagnostic techniques and therapeutic strategies.

**Disclosure of Interest:** None declared.

#### O054

##### **In Vivo B cell depletion with rituximab reduces the incidence of post-transplant lymphoproliferative disorders after allogeneic stem cell transplantation: a single centre experience between 2000 and 2015**

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**Introduction:** Post-transplant lymphoproliferative disorders (PTLD), ranging from reactive polyclonal hyperplasia to high grade lymphomas, are rare but potentially fatal complications of solid organ (SOT), autologous and allogeneic hematopoietic stem cell transplantation (HSCT). Occurring in 1-20% of recipients, the incidence depends on transplant type (SOT>HSCT) and risk factors: alternative donor, recipient's age, sieronegative recipient of sieropositive graft, T cell depletion, ATG, graft versus host disease, immunosuppression. Usually associated with T cell dysfunction and the Epstein-Barr virus, no standard therapy exists for PLTD. Best treatments are reduced immunosuppression, ab anti CD20+ (Rituximab) as pre-emptive therapy (viral load >1000 genome/ml), EBV specific cytotoxic cell line infusions, chemo-radiotherapy for monomorphic lesions. Since few data are available on PTLD prophylaxis with rituximab, we retrospectively investigated whether *in vivo* B cell depletion with Rituximab in the peri-transplant period impacted on the incidence of PTLD and transplant outcomes after HSCT.

**Material (or patients) and methods:** Between 2000 and 2015, 600 recipients of allo-HSCT after myeloablative conditioning were enrolled. 2000-2010: No PLTD prophylaxis was given. 2010-2015: patients received *in vivo* B cell depletion with Rituximab 500 mg day 0 if CD20+ B cells were  $>1 \times 10^5$ /kg. The median number of infused B cells was  $4.3 \times 10^5$ /kg. All recipients were screened by quantitative PCR for EBV DNA weekly until day 100 or until reaching  $>200$  CD4+ T cells.

**Results:** 2000-2010: PTLD developed in 13/376 patients (11 recipients of T depleted haplo-HSCT, 2 of T depleted matched transplants). Incidence was 3.4%. Two died (0.55%). Median time from transplant was 4.2 months (range:1.5-10). Median EBV viral load at diagnosis was 9590 genomes/ul (range 106-68.387). 2011-2015: PTLD developed in 4/224 patients (1 recipient of T depleted haplo-HSCT, 2 of haplo-HSCT with regulatory and conventional T cell adoptive immunotherapy, 1 MUD transplant). Incidence was 1.8%. One patient died (0.44%). Median time from transplant was 4.5 months, (range: 3-8), median EBV viral load at diagnosis was 3.500 genomes/ul (range 1137-46.0000).

**Conclusion:** In vivo depletion of host and donor B cells significantly reduced the incidence of PTLD post-HSCT. Rituximab is effective as pre-emptive therapy for increased viral load and for PTLD treatment. Although it delays post-transplant B cell reconstitution for up to 1 year, administration in the peri-transplant period impacted less on B cell recovery.

**Disclosure of Interest:** None declared.

**O055**

**Human herpesvirus type 6 reactivation after haploidentical hematopoietic stem cell transplantation with post-graft cyclophosphamide is associated with graft-versus-host disease and decreased risk of relapse**

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**Introduction:** Human herpesvirus type 6 (HHV6) reactivation is common after conventional allo-SCT. However, its impact on patient outcome has never been assessed in a haploidentical (haplo) setting with the use of post-graft high dose cyclophosphamide.

**Material (or patients) and methods:** HHV6 plasma loads were monitored weekly by quantitative PCR in 49 consecutive patients who underwent haplo SCT in our transplant unit. The stem cell source was peripheral blood stem cells (n=29; 59%) and bone marrow (n=20; 41%). Conditioning regimen consisted in thiotepa – busulfan – fludarabine (TBF; n=26), sequential conditioning combining thiotepa – etoposide – cyclophosphamide (n=15) or clofarabine-cytarabine (n=2) followed by RIC regimen with fludarabine – busulfan and Thymoglobuline (FB2-SAL), and FB2-SAL alone (n=6). Thymoglobuline was administered to 39 patients. GVHD prophylaxis was cyclosporine and mycophenolate mofetil in all patients. High dose cyclophosphamide was administered on D+3 (n=49) and D+5 (n=29).

**Results:** Median age was 45.2 years (range 16-72). Patients were treated for AML (n=25), ALL (n=11), MDS/MPN (n=7) and lymphoma (n=6). Median follow-up was 22 months (range, 2-36). HHV6 reactivation occurred in 32 patients (65%) at a median time of 18 days post-transplant (3-122). There was no significant difference between HHV6+ and HHV6- patients in terms of age, sex mismatch, disease risk, disease status at transplant, type of graft, conditioning regimen or use of Thymoglobuline. All 49 patients experienced neutrophil engraftment after a median time of 17 days (12 - 88), with no significant difference according to HHV6 reactivation. HHV6 + patients experienced more often secondary thrombopenia, which required thrombopoietin in 11/32 patients compared to 1/17 HHV6- patients. Accordingly, there was a high association between the reactivations of HHV6 and CMV (21 in HHV6+ vs. 10 in HHV6- patients; P < 0.001) and/or EBV (20 in HHV6+ vs. 11 in HHV6- patients; P < 0.001). HHV6+ patients experienced grade 1-3 hemorrhagic cystitis with positive BK virus in 44% of cases compared to 29% HHV6- patients (P < 0.001). Two patients required specific antiviral therapy for HHV6: 1 with encephalitis and 1 with extended rash associated with hepatic cytolysis. HHV6 reactivation occurred before the development of acute GVHD in 27/32 HHV6+ patients. Previous HHV6 reactivation was associated with a higher risk of grade II-IV acute GVHD (44.4% in HHV6+ patients vs. 11.8% in HHV6- patients). It is noteworthy that in HHV6- patients no grade III-IV or corticoreistant acute GVHD were observed, whereas 4 HHV6+ patients developed grade III-IV acute GVHD. HHV6 reactivation was also associated with chronic GVHD (46% in HHV6+ vs. 15% in HHV6-; P=0.041). In terms of outcomes, HHV6 reactivation was associated with decreased risk of relapse (9.4% vs. 59%; P < 0.0001) and improved 2-year event-free survival (62.5% vs. 23.5%; P=0.011). 2-year non-relapse mortality was 28% and 17.5% (P=0.515) and 2-year overall survival was 62.5% and 47% (P=0.365) in HHV6+ and HHV6- patients, respectively.

**Conclusion:** HHV6 reactivation seems to occur frequently after haploidentical SCT. In contrast with most reports in conventional allo-SCT, HHV6 is associated with a higher risk of chronic GVHD and better outcome in terms of relapse and event-free survival.

**Disclosure of Interest:** None declared.

**O056**

**Centralized patient reported quality of life collection in hct patients is feasible, and higher pre-HCT scores significantly predict better overall survival post-transplant**

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**Introduction:** Patient Reported Quality of Life (QoL) is predictive of outcomes in allogeneic hematopoietic cell transplant (aHCT) recipients. Routine collection of QoL measures in patients contributing data to large outcome registries is not standard. The primary aim of this pilot project was to evaluate the feasibility of prospective collection of QOL data on aHCT recipients in multiple transplant centers (TC) reporting clinical data to the Center for International Blood and Marrow Transplant Research (CIBMTR). A secondary aim was to correlate QOL data with clinical and demographic data routinely collected.

**Material (or patients) and methods:** 373 consecutive patients (263 adults, 84 children) were enrolled at 8 US TCs. Participants completed QoL measures at four time points: pre-HCT, day 100, 6 months and 1 year (adults: FACT-BMT and SF36; children/parents: age appropriate and proxy PedQL modules). Baseline forms and consent occurred at the TC, while all later contact was with CIBMTR by phone or mail. Pre-transplant, treatment variables and clinical outcomes were collected as routine on CIBMTR report forms. Additional socio-demographic variables were collected at baseline. A satisfaction survey was completed by participants at 12 months. Binomial regression was used for the feasibility analysis, where feasibility was defined as the proportion of forms completed out of the total possible number. Cox proportional hazards modeling was performed to evaluate associations between pre-HCT QoL and overall mortality after adjusting for factors associated with transplant outcomes.

**Results:** At the majority of centers > 60% of screened patients agreed to participate (31-85%). Time point retention rates were 69%, 75.8% and 73.9% at day 100, 6 months and 1 year respectively. Factors negatively associated with participation in adults were race other than Caucasian (OR 0.21; CI 0.12-0.36; P < 0.0001) and unmarried status (OR 0.41; CI 0.25-0.65; P=0.0002). In children, higher family income (OR 4.72; CI 1.98-11.25; P=0.0005) was positively associated with participation. 90% of patients reported that they would be willing to complete QoL measures in the future. Scores at baseline for the FACT-BMT, Physical Component Score (PCS) of the SF36 and the PedQL were predictive of overall survival (table 1). In adult patients, older age and the use of cord blood were the only other factors significantly associated with survival, while for children no other significant factors were found.

	Score (higher is better)	RR of mortality (95%CI)	P-value
FACT-BMT (adult)	10 point increase	0.83 (0.75 - 0.92)	0.0003
PCS of SF36 (adult)	10 point increase	0.78 (0.64 - 0.95)	0.0147
PedQL (child)	10 point increase	0.69 (0.48 - 0.99)	0.0444
PedQL (parent)	10 point increase	0.79 (0.60 - 1.03)	0.0816

**Conclusion:** Our results show that it is feasible to prospectively collect QoL measures at multiple time points for unselected transplant patients with an excellent return rate and high levels of patient satisfaction. Importantly, we found that baseline QoL scores are significantly predictive of survival adjusting for clinical factors; suggesting that patient reported outcomes add unique prognostic information not captured by current routine data collection. In addition, they may have a role as an adjustment factor in certain transplant analyses.

**Disclosure of Interest:** None declared.

**O057**

**Cryotherapy in adult patients with myeloma treated with high-dose melphalan and autologous stem cell transplant: preliminary results of randomized study**

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**Introduction:** The Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology guidelines only suggest the use of oral cryotherapy to prevent oral mucositis in patients receiving high-dose melphalan, with or without total body irradiation, as conditioning regimen for autologous hematopoietic stem cell transplant (ASCT). However, a strong recommendation was not possible from these guidelines, due to inadequate evidences derived from published studies. Several of these studies were performed some years ago and based on patients with myeloma treated before ASCT with conventional anthracycline-based chemotherapy. Based on these considerations, we aimed to perform a prospective randomized study to establish: 1. If cryotherapy prophylaxis is able to prevent grade 3-4 oral mucositis in myeloma patients who underwent ASCT after a chemo-free induction treatment; 2. If cryotherapy prophylaxis is able to reduce the incidence of febrile episodes and documented infections in this setting of patients.

**Material (or patients) and methods:** From October 2013 to September 2015, 65 consecutive adult myeloma patients who underwent an ASCT after conditioning regimen with high-dose melphalan (200 mg/m<sup>2</sup>) after a chemo-free induction treatment (median lines of pre-transplant treatment: 1, range 1-3) were enrolled in this study. Patients were randomly assigned to receiving (n = 33) or not (n = 32) prophylaxis of oral mucositis by the use of local cryotherapy during the administration of chemotherapy. All patients were treated with the same hydro-electrolytic support therapy, anti-infectious and transfusion policy. All patients underwent

administration of filgrastim 5 mcg/Kg starting on day +3 from stem cell infusion as febrile neutropenia prophylaxis until absolute neutrophils recovery (>500/mcL for 3 consecutive days). Oral mucositis was assessed with daily physical examination and reported in medical records, using Common Toxicity Criteria (version 4.02). All patients had signed an informed consent granting use of sensitive data for scientific purposes.

**Results:** We observed a statistically significant decrease of any grade and grade 3-4 oral mucositis in the group of myeloma patients who received cryotherapy [18.2% vs 53.1% (P=0.031) and 6.1% vs 37.5% (P=0.002), respectively]. As a consequence, in this group we observed a significant reduction of intravenous opioids need for pain control (3% vs 28.1%; P=0.006) and of total parenteral nutrition for significant (>10%) weight loss (6.1% vs 32.3%; P=0.011). Interestingly, patients assigned to cryotherapy group showed a trend toward a lower incidence of documented infectious episodes (21.2% vs 40.6%; P=0.112) and a significant lower rate of patients needing intravenous antibiotics was observed into this group (27.3% vs 53.1%; P=0.044). Transplant-related mortality, neutrophils and platelets recovery and days of hospitalization were similar between the two groups.

**Conclusion:** Our preliminary results show that local cryotherapy during high-dose melphalan administration in myeloma patients who underwent an ASCT after a chemo-free induction treatment is able to significantly decrease the incidence of oral mucositis. Moreover, patients treated with cryotherapy seems to have a lower risk of treatment with intravenous antibiotics for suspected or documented infections during the engraftment phase.

**Disclosure of Interest:** None declared.

**O058**

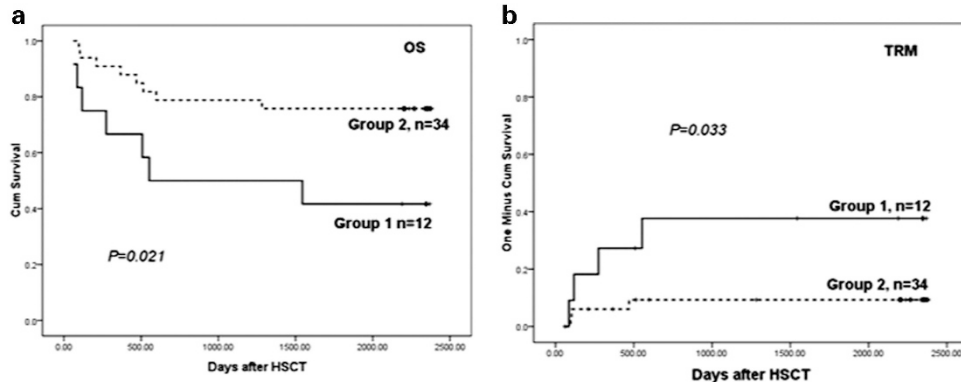
**Characterization of thrombopoietin kinetics within 60 days after allogeneic hematopoietic stem cell transplantation and its correlation with megakaryocyte ploidy distribution**

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**Introduction:** Thrombopoietin (TPO) has been identified as a key cytokine for both megakaryogenesis and thrombopoiesis, and it may be associated with thrombocytopenia after allogeneic hematopoietic stem cell transplantation (allo-HSCT). We attempt to characterize the kinetics of TPO and its correlation with megakaryocytes (MKs) ploidy distribution pattern within 60 days after allo-HSCT.

**Material (or patients) and methods:** Forty-six consecutive patients undergoing allo-HSCT from October 2008 to December 2008 were included. Thirteen healthy volunteers were chosen as healthy control subjects. TPO levels and ploidy distribution patterns of MKs were measured using ELISA and

**[0058]**



**Figure 1.** The endogenous TPO levels on day 60 are associated with transplant outcome after allo-HSCT including OS (A) and TRM (B). Group 1: patients with endogenous TPO levels >250 pg/ml on day 60 (n=12); Group 2: patients with endogenous TPO levels ≤250 pg/ml on day 60 (n=34).

flow cytometric analysis, respectively. Blood samples for TPO tests were drawn from patients at the following time points: on the day prior to conditioning as well on day 0, day 15, day 30, day 45, and day 60 after allo-HSCT. But for thrombocytopenic patients with platelet counts lower than  $20 \times 10^9/L$ , who were platelet transfusions dependent, the samples for TPO tests might be obtained 1-3 days before or after the days in protocol. As the samples were collected before platelet transfusions to avoid the effect of transfusions, and the platelet counts before platelet transfusions were recorded as their actual platelet levels. However, for patient with refractory platelet transfusions, who needed daily platelet transfusions, the samples were collected on the days in protocol. On day 60, bone marrow was collected.

**Results:** The results indicated that TPO levels and the platelet count followed opposite trends after allo-HSCT. The preconditioning TPO levels and the number of transplanted CD34<sup>+</sup> cells were significant predisposing factors for rapid platelet engraftment ( $P=0.010$  and  $0.007$ , respectively) by multivariate analysis. There was a reduction of ploidy and an increase in immature MKs in patients with higher endogenous TPO levels ( $>250$  pg/ml) on day 60 after allo-HSCT. Moreover, lower TPO levels ( $\leq 250$  pg/ml) on day 60 after allo-HSCT were associated with significantly improved 5-year overall survival ( $P=0.021$ ) and reduced transplant-related mortality ( $P=0.033$ ).

**Conclusion:** Endogenous TPO levels may be associated with platelet recovery and have prognostic significance during allo-HSCT.

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**Disclosure of Interest:** None declared.

#### O059

##### **Geriatric assessment and quality of life in patients above 60 years considered for allogeneic stem cell transplantation: a prospective risk factor and serial assessment analysis in 108 patients**

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**Introduction:** Hematological malignancies affect mainly patients above 60 years, an age group considered "elderly" in hematology. To estimate the risk and benefits of a potentially curative treatment option such as hematopoietic cell transplantation (HCT) while frequently facing limitations in physical or mental reserves is a difficult medical task. Comprehensive Geriatric and Quality of Life Assessment (CGA/QOL) provides detailed information on critical aspects. A relevant question is whether CGA/QOL can be used to stratify which elderly patients will fare well with HCT and how patients recover with regards to functionality (independence) and subjective QOL.

**Material (or patients) and methods:** This was a prospective prognostic factor study in patients  $\geq 60$  years undergoing allogeneic HCT at the University of Freiburg, Germany. CGA including a battery of 8 validated instruments and QOL (EORTC QLQ C-30) was performed pre-HCT, planned at day +30, +100, and +180. All 108 consecutive patients  $\geq 60$  years admitted to the Transplant Unit for allogeneic HCT who met the inclusion criteria were enrolled. To investigate whether CGA had a prognostic value for progression free survival (PFS) and overall survival (OS), Cox models were calculated. Changes of CGA parameters in serial post HCT analyses were assessed to describe recovery of CGA/QOL variables.

**Results:** Median follow-up of 108 patients was 43.5 months, median age 66 years (range 60-78). 62% of patients were male. With the exception of 6 patients, all had myeloid malignancies with  $n=19$  (17.6%) in CR1;  $n=88$  (82%) had advanced disease risk at HCT. The majority (85.2%) was treated according to the FBM protocol.<sup>(1)</sup> Median Karnofsky Index (KI) was 80 (40-100) and median HCT-CI 2 (0-7). Ten patients (9.3%) had activities of daily living (ADL) scores  $<100$  and  $n=33$  (30.6%) had impairments in instrumental ADLs. Median PFS was 13.4 months with 38.3% alive at 2 years and median OS was 16.3 months with 43% alive at 2 years. The following risk factors were predictive for PFS and OS, respectively. Age: HR 1.085 ( $P=0.0007$ ) and HR 1.09 ( $P=0.0008$ ); HR 0.957 for a ten point change in QOL role function ( $P=0.24$ ) and HR 0.926 ( $P=0.045$ ); and fatigue HR 1.007 ( $P=0.08$ ); HR 1.01 ( $P=0.02$ ); KI: HR 0.98 ( $P=0.046$ ); HR 0.97 ( $P=0.01$ ). HCT-CI resulted in HR 1.12 ( $P=0.057$ ); HR 1.1 ( $P=0.069$ ), respectively. Serial CGA/QOL revealed a significant decline in all functional and QOL dimensions at d+30. In d+180 survivors, mean values re-approached baseline results.

**Conclusion:** Results indicate the potential prognostic value of CGA/QOL applied to older, high-risk HCT recipients as an important additional tool in clinical evaluations. Physician-assessed KI was a stronger predictor of outcome than self-reported ADL while impaired role function and fatigue underscored the importance of patient reported measures for further validation studies. After a decline of all variables at d+30, patients can be ascertained that full recovery of independence and QOL is achieved in the vast majority of survivors.

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**Disclosure of Interest:** None declared.

#### O060

##### **The hematopoietic cell transplantation comorbidity index (HCT-CI) predicts outcomes in patients with acute myeloid leukemia and myelodysplastic syndromes receiving CD34+ selected grafts for allogeneic hematopoietic cell transplantation**

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**Introduction:** The HCT-CI has been validated as a prognostic tool in several allo-HCT settings. However, it has not been studied in CD34-selected allo-HCT, an approach shown to significantly reduce GVHD and which is now under investigation in a phase 3 study (BMT CTN 1301, NCT02345850). Moreover, the age-adjusted HCT-CI (HCT-CI/age) has not been validated in large and independent cohorts.

**Material (or patients) and methods:** All consecutive patients with acute myeloid leukemia (AML) in complete remission or myelodysplastic syndrome (MDS) with  $\leq 5\%$  blasts at HCT undergoing CD34-selected allo-HCT from peripheral blood mobilized progenitors in our center from 2001 to 2013 were included. HCT-CI and HCT-CI/age were calculated as originally defined. The c-statistic was used to determine the predictive capacity of each model.

**Results:** 346 patients (AML = 244, MDS = 102) were included. Median age at HCT was 56 years (range 18-73). Median follow-up for survivors was 57 months (range 12-164). Most frequent comorbidities included pulmonary impairment (moderate [ $n=45$ , 13%], severe [ $n=108$ , 31%]), psychiatric disorders ( $n=70$ , 20%) and hepatic impairment (mild [ $n=54$ , 16%], moderate/severe [ $n=7$ , 2%]). Median HCT-CI score was 2

[0060]

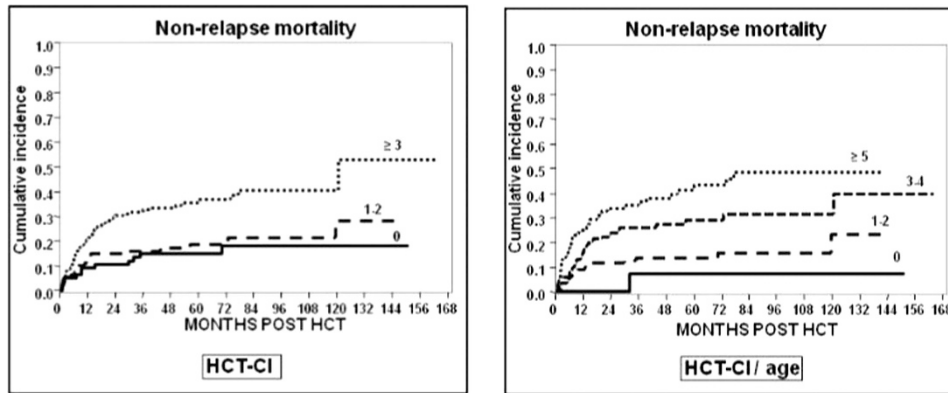


Figure 1. Cumulative incidence of NRM according to the HCT-CI and HCT-CI/age.

(percentile 25-75: 1-4). According to the HCT-CI, 76 patients (22%) had a score of 0, 108 (31%) a score of 1-2 and 162 (47%) had a score  $\geq 3$ . According to the HCT-CI/age, median score was 3 (percentile 25-75: 1-5) and distribution was as follows: HCT-CI/age=0 ( $n=15$ , 4%), 1-2 ( $n=122$ , 35%), 3-4 ( $n=117$ , 34%),  $\geq 5$  ( $n=92$ , 27%).

Cumulative incidence of non-relapse mortality (NRM) for the whole cohort at 100 days and 3 years was 6.6% (95%CI 4-9.6) and 23.5% (95%CI 19.2-28.2), respectively. The probability of overall survival (OS) and relapse-free survival at 3 years was 59% (95%CI 54-64) and 56% (95%CI 51-62), respectively. Probability of CRFS at 1 year (chronic GVHD relapse-free survival, BMT CTN 1301 primary endpoint) was 69% (95%CI 63-73). Using the HCT-CI, NRM (Figure) and OS (not shown) were similar for patients scoring 0 and 1-2, while they were significantly worse for patients with HCT-CI  $\geq 3$ . For the HCT-CI/age, there was a progressive increase in risk of NRM and a decrease in OS (not shown) with increasing scores. In particular, there was about 10% incremental decrease in OS in all 4 risk-groups, ranging from 86% (score = 0) to 45% (score  $\geq 5$ ) at 3 years ( $P < 0.001$ ). Both increased HCT-CI and HCT-CI/age had an association with higher risk of NRM ( $p < 0.001$ ) and lower risk of OS ( $p < 0.001$ ). The c-statistic for the HCT-CI and the HCT-CI/age scores for NRM were 0.616 and 0.647, respectively. Similarly, the higher HCT-CI and HCT-CI/age scores were associated with lower CRFS ( $P=0.001$ ). None of the indexes showed an association with relapse.

**Conclusion:** Similar to analyses in recipients of unmodified HCT, the HCT-CI and HCT-CI/age are associated with transplant outcomes in CD34-selected allo-HCT, including NRM, OS and CRFS. Interestingly, patients with HCT-CI of 0 and 1-2 have similar low NRM suggesting that, with ablative conditioning, CD34-selected PBSCT may have lower toxicity than unmodified HCT.

**Disclosure of Interest:** None declared.

0061

**Posterior reversible encephalopathy syndrome (PRES) after allogeneic stem cell transplantation in pediatric patients with Fanconi anemia**

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**Introduction:** Posterior reversible encephalopathy syndrome (PRES) is a serious neurologic complication following hematopoietic stem cell transplantation (HSCT). This prospective study assesses the incidence, clinical associations and imaging features of PRES in post-HSCT pediatric patients with Fanconi anemia in comparison with other diseases.

**Material (or patients) and methods:** All Pediatric patients who underwent HSCT between March 2014 and September 2015 in our center were enrolled. We carried out brain MRI in all patients who developed neurologic symptoms/signs. In patients with clinic-radiologic diagnosis of PRES, a follow-up MRI was conducted in two months. Based on the primary disease before the transplant, patients were divided into two groups of Fanconi anemia or non-Fanconi-anemia.

**Results:** A total of 170 pediatric patients aged  $< 15$  years (22 with fanconi anemia and 148 with non-fanconi anemia diseases) underwent HSCT in our center using TBI-free conditioning regimens mainly according to patients' disease and transplant center protocols. Busulfan/cyclophosphamide was the most conditioning regimen used, as low-dose busulfan and cyclophosphamide were used in pediatrics with Fanconi anemia. Seizure was the most common neurologic symptom followed by headache. Of all the individuals with neurologic symptoms, brain MRI revealed PRES in 16, of which 6 were the ones with Fanconi anemia (6/22, 27.2%) and 10 were the patients with other diseases (10/148, 6.75%). All patients with PRES had received cyclosporine as graft-versus-host disease prophylaxis regimen. In Fanconi anemia group, Micro-hemorrhagic foci were depicted in MRI of 2 patients, while in one patient PRES progressed to infarct. Follow-up MRI showed persistent imaging findings in these three patients. Whereas, in non-Fanconi anemia group, 2 patients had microhemorrhagic PRES with one of them being persistent in follow-up study. Of 16 patients with PRES, 2 (33.3%) individuals in Fanconi anemia group (one being microhemorrhagic PRES) and 2 (20%) in non-Fanconi anemia group died during the follow up period.

**Conclusion:** Our results showed that pediatrics with Fanconi anemia are more prone to PRES than other diseases following HSCT, which might be caused by genetic defect. PRES might be associated with increased mortality rate in these pediatric patients. Multi centric prospective study is recommended in this issue.

**Disclosure of Interest:** None declared.

0062

**Intrabone injection of hematopoietic stem cells (IB-HSC) to treat poor graft function (PGF) after allogeneic hematopoietic stem cell transplant (HSCT)**

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**Introduction:** PGF is a life-threatening HSCT complication. Various approaches potentially improve PGF, such as

stimulating factors, second HSCT or HSC boost. Based on data showing high rate of engraftment and low acute GVHD with IB-HSC, in particular IB-cord blood, we performed IB-HSC boost to treat PGF in patients (pts) missing an adequate dose of donor HSC, when a second harvest/mobilization was not safe.

**Material (or patients) and methods:** We treated 10 pts with IB-HSCT boost for primary (5 pts) or secondary (5 pts) PGF from 2009 to 2015. The IB infusion was performed under local anaesthesia and with short conscious sedation, in the BMT ward.

4 pts suffered from AML, 3 MPN, 1 CMML, 1 HL, 1 Burkitt Lymphoma. 7 pts received a previous HSCT from haploidentical donors, 2 pts from MUD (9/10 and 10/10 HLA-matched), 1 pt from an HLA-matched related donor. Median age was 52 (range 20-63).

4 pts received a BM boost, harvested from donors the same day under sedation, neither requiring general anaesthesia, nor operating theatre. 6 pts received cryopreserved PBSC. In 2 cases the PBSC were CD34+ selected. No washing and no dilution were used.

At the boost, 9 pts were on immunosuppressant (Rapamycine, MMF or Cyclosporine), all had full donor chimerism. The median neutrophil (ANC) count was  $0.6 \times 10^9/L$  (range 0, to 1.5), the median platelet (PLT) count was  $16 \times 10^9/L$  (range, 5 to 50), and the median Hb was 8.5 g/dL (range, 7.3 to 9.5).

Hematological improvement (HI) was defined as  $ANC > 1.5 \times 10^9/L$ ,  $PLT > 30 \times 10^9/L$ , and  $Hb > 8.5 g/dL$ , based on minimal values not requiring transfusions and associated with reduced risk of infections. Hematological response (HR) was defined as  $ANC > 2.5 \times 10^9/L$ ,  $PLT > 100 \times 10^9/L$ , and  $Hb > 10 g/dL$ .

**Results:** The median time after HSCT was 80 days (range, 31 to 205). The median CD34+ cells were  $1.26 \times 10^6/kg$  b.w. (range, 0.23 to 3.58). The median CD3+ T cells were  $1.6 \times 10^7/kg$  b.w. (range, 0 to 44.5). The IB-HSC was performed mono- or bilaterally, according to the volume infused. The median BM volume was 140 ml (range, 88 to 170). The median PBSC volume was 90 ml (range, 40 to 120).

No immediate or late adverse reactions were observed. 2 pts developed acute GVHD, and they were the only ones receiving CD3+ T cells  $> 1 \times 10^8/kg$  b.w.

HI was evaluable in 9 pts (1 pt died 11 days post-boost). All 9 achieved  $ANC > 1.5 \times 10^9/L$  after a median of 12 days (range, 5-71) and 6 achieved  $ANC > 2.5 \times 10^9/L$  (median 15 days; range, 10-208). 5 achieved  $PLT > 30 \times 10^9/L$  at a median of 14 days (range, 0-32) and 3 achieved  $PLT > 100 \times 10^9/L$

(median 172 days; range, 131-289). 3 achieved  $Hb > 8.5 g/dL$  at a median of 62 days (range, 16-71) and  $Hb > 10 g/dL$  (median 110 days; range, 96-115).

3 pts obtained a complete HR, 1 patient received a second boost and reached a complete HR. At last follow-up those 4 pts are alive and in CR (median follow-up 58 months; range, 4.3-74.5).

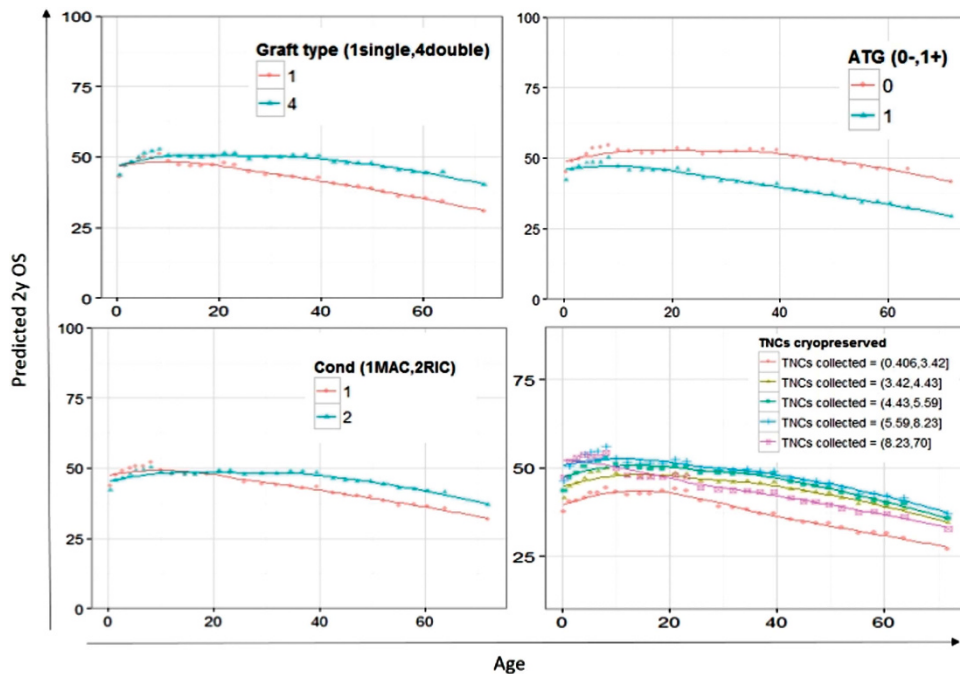
**Conclusion:** IB-HSC boost without conditioning chemotherapy is feasible, safe, easy to perform and improves engraftment (particularly ANC engraftment) and survival outcome. This procedure allows an early and timely HSC boost, avoiding donors a second PBSC mobilization and/or BM harvest. Even when not completely successful IB-HSC could be a pivotal bridge to further PGF treatments. This is of paramount importance when a prompt improvement of blood counts is necessary to control or prevent potentially fatal bleedings and infections.

**Disclosure of Interest:** None declared.

### O063 Identifying Overall Survival Predictors and Related Interactions in Umbilical Cord Blood Transplantation Using Random Survival Forests: A Eurocord - Acute Leukemia Working Party - Paediatric Diseases Working Party - EBMT STUDY

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[O063]



**Introduction:** Clinical benefit of umbilical cord blood transplantation (UCBT) varies among acute leukemia patients, and is likely the result of a complex interplay between donor, recipient, and procedural characteristics. The study's objective was prediction of overall survival (OS) at 2 years following UCBT, while ranking variables' predictive contribution and analyzing potential interactions. Random Survival Forest (RSF), a machine learning modeling technique, enabling recovery of non-linear associations, was used for the statistical analysis.

**Material (or patients) and methods:** A cohort of 3,140 UCBT were analyzed. Inclusion criteria encompassed patients at all ages, undergoing an UCBT (single/double unit) in European Society for Blood and Marrow Transplantation (EBMT) centers, from 2004 to 2014, for acute leukemia, in all disease status. Nineteen variables were considered. The analysis pipeline was compromised of sequential stages: 1. Preprocessing- data quality assurance and multiple imputations of missing values; 2. Development and validation of a non-parsimonious (i.e., using all variables) prediction models using RSF; 3. RSF variable importance estimation; 4. RSF Interaction analysis using conditional plots (cplots), which provide intuitive visualization of how an outcome depends on two or more variables.

**Results:** The 2 year OS was 53% with a median follow-up of 30 months. Non-parsimonious RSF models developed on 20 derivation datasets, generated through multiple imputations of missing values, reached an average (standard deviation) C-index of 63.75% (+/-0.32). Similar performance was noted for corresponding validation sets (C-index = 63.55% (+/-0.52)). The top 8 predictors selected by RSF were disease status, age, number of total nucleated cells (TNCs) cryopreserved, anti-thymocyte globulin (ATG) administration, transplant year, center experience, recipient's cytomegalovirus (CMV) serostatus, and the interval from diagnosis to UCBT. Importance was verified by constructing nested RSF models for 2 years OS, developed on serially introduced ranked variables. Indeed, performance plateaued when presented with the top 8 variables described. Advanced disease status, ATG administration, CMV sero-positivity, and increasing age were associated with worse outcomes. Increasing center experience and number of TNCs resulted in improved outcome until reaching a plateau. Selected interactions discovered (figure) were a non-linear relationship between increasing age and worse predicted survival in patients receiving ATG, single CB units, and myeloablative conditioning (MAC). In addition, though a modest effect, double CB units could overcome the detrimental impact of a low TNC dose, as opposed to single units, where TNCs number had a more prominent role. Finally, despite the absence of a direct interaction, patients receiving more than  $3.4 \times 10^6$  TNCs/kg had superior 2 year OS compared to those receiving lower numbers.

**Conclusion:** The Random Survival Forest algorithm proved useful in providing a ranked list of 2 year overall survival predictors. Moreover, it captured clinically relevant interactions. Our findings highlight the need for personalizing UCBT, as benefits varied among subpopulations.

**Reference:** Ishwaran, H., et al., *Random survival forests*. The Annals of Applied Statistics, 2008: p. 841-860.

**Disclosure of Interest:** None declared.

## O064

### Randomized Trial Comparing R-chop-14 Versus High Dose Sequential Chemotherapy in High Risk Patients with Diffuse Large B-cell Lymphomas. Final results from the GITIL-RHDS0305 study

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**Introduction:** The role of high dose chemotherapy with autologous stem cell transplantation remains unclear as front line treatment of high-risk Diffuse Large B-Cell Lymphoma (DLBCL) patients.

**Material (or patients) and methods:** The cooperative study group Gruppo Italiano Terapie Innovative dei Linfomi (GITIL) designed an open-label, multicenter randomized, phase III trial to compare R-CHOP-14 (8 cycles) to a Rituximab supplemented, high dose sequential chemotherapy program (R-HDS) with autologous stem cell transplantation (1). (Clinicaltrials.gov NCT00355199). Inclusion criteria were: age 18-65 years, a biopsy-confirmed diagnosis of CD20 positive DLBCL, advanced Ann Arbor stage without CNS involvement, no previous treatment. All patients were in an intermediate-high or high risk group by IPI. The primary endpoint was the event free survival (EFS); secondary endpoints were: the response rate, the progression-free survival (PFS), disease-free survival (DFS), overall survival (OS) and toxicity. Analysis of primary outcome was intention to treat.

**Results:** From June 2005 to June 2011, 246 DLBCL with the following features were randomized: median age 51 years (range 19-66 years), males 58%, Ann Arbor stage III-IV (92%), elevated LDH (86%), ECOG-PS > 1 (62%),  $\geq 2$  extranodal sites (43%), bone marrow infiltration (22%), bulky disease (68%), B-symptoms (59%). Risk score evaluation was high-intermediate in 56% of cases and high in 44%. The two arms were well balanced with respect to all presenting features. In R-CHOP or R-HDS respectively, the planned treatment was discontinued due to different reasons in 6 (5%) vs. 22 patients (19%) (toxicities 4 vs. 17; medical (1 vs. 2) or patient decision (1 vs. 3)). The final autograft in R-HDS was performed in 83/116 (72%), with a median of  $7.2 \times 10^6$ /kg CD34+ cells (range 3-30 x  $10^6$ /kg). No graft failures were reported. The response to R-CHOP as compared to R-HDS therapy was not significantly different. After a median follow-up of 5 years (range 0.02-9.49), the 3-year EFS was 62% (95% CI: 55% - 72%) for patients treated with R-CHOP vs. 66% (95% CI: 57% - 75%) for patients



treated with R-HDS ( $P=0.74$ ), (HR 0.96, 95% CI 0.64–1.44); the PFS was 66% in R-CHOP (95% CI: 58% - 74%) vs. 75% (95% CI: 68% - 84%) ( $P=0.097$ ) after R-HDS. Interestingly, the 3-years DFS was better in the experimental arm being 79% (95% CI: 72% - 88%) vs. 91% (95% CI: 85% - 97%) in R-CHOP and R-HDS respectively ( $P=0.036$ ). No difference was found in terms of OS, being 74% (95% CI: 67% - 82%) in R-CHOP vs. 78% (95% CI: 71% - 86%) in R-HDS ( $P=0.55$ ). Hematologic toxicity (grade III-IV) was higher in the experimental arm: neutropenia 34% vs. 84% ( $P < 0.0001$ ), anemia 14% vs. 71% ( $P < 0.0001$ ), thrombocytopenia 5% vs. 85% ( $P < 0.0001$ ) in the R-CHOP vs. R-HDS, respectively. Patients in the R-CHOP arm had less GI tract toxicity (10% vs. 29%,  $P < 0.0001$ ) and lower frequency of severe infections (9%, vs. 53%,  $P < 0.0001$ ).

**Conclusion:** In a multicenter setting, the feasibility of R-HDS was more difficult compared to R-CHOP-14 and both programs proved equally, highly effective in high-risk DLBCL patients.

**Reference:** (1) Tarella C. et al. *Leukemia*, 2007;21(8):1802-1811

**Disclosure of Interest:** None declared.

## O065

### Allogeneic stem cell transplantation for pediatric patients with very high risk acute lymphoblastic leukemia in 2003 BFM and 2007 International-BFM studies: impact of donor type on the outcome

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**Introduction:** A broader degree of HLA mismatching may be considered for ALL pediatric patients at highest risk profile eligible for transplantation but lacking a matched donor. Between 2003 and 2011, 1119 patients, 18 years of younger, affected with ALL in CR were enrolled in either the ALL SCT 2003 BFM Study ( $n=708$ ) or the ALL SCT 2007 Int BFM Study ( $n=411$ ). Besides the primary aim of assessing whether the outcome of HSCT from MD (10/10 or 9/10 4 digit matched donor) was not inferior to the outcome of HSCT from MSD (HLA-identical sibling), the secondary aim was to assess the outcome of MMD versus MD HSCT, with EFS and non-relapse mortality (NRM) as primary endpoints.

**Material (or patients) and methods:** Patients were stratified by disease severity: only the 541 (63% male, median age 10 ys) at very high risk (VHR) and therefore eligible for transplant

from any donor, including MMD (98), other than MSD/MD (443), are described here.

The 2 groups were not balanced for disease phase (more CR > 2 in MMD,  $P=0.04$ ), stem cell source (more PBSC and CB in MMD group,  $P < 0.0001$ ; 19% of MMD-patients received UCB) and donor age (older in MMD group,  $P < 0.0001$ ). Conditioning regimen per protocol consisted of TBI-VP16 for all patients > 2 ys and Bu-Cy-VP16 for < 2 ys. GVHD prophylaxis consisted of CSA alone for patients transplanted from MSD, ATG+CSA+MTX for MD recipients and varied for MMD recipients, with T-cell depletion performed in 44% of the cases. Median follow-up was 4.5 years.

**Results:** For MMD and MSD/MD recipients 4-yr OS was  $43 \pm 6\%$  and  $60 \pm 2\%$  ( $P < 0.001$ ), EFS  $41 \pm 6\%$  and  $69 \pm 2\%$  ( $P < 0.001$ ), 4-yr CIR  $36 \pm 5\%$  and  $30 \pm 2\%$  ( $P=NS$ ), 4-yr NRM  $24 \pm 4\%$  and  $9 \pm 1\%$  ( $P < 0.001$ ), respectively. Differences in OS, EFS, and NRM remain statistically significant in the subgroups of patients transplanted in CR1 and CR2.

Grade II-IV and III-IV aGVHD occurred in 30% and 11% of MMD vs 35% and 12% of MD recipients, respectively.

Among patients transplanted in CR1,  $4 \pm 4\%$  and  $16 \pm 7\%$  of MMD recipients and  $10 \pm 2\%$  and  $10 \pm 2\%$  of MSD/MD recipients developed limited and extensive cGVHD ( $P=NS$ ). There were no significance neither regarding for patients transplanted in CR2.

In multivariate analysis, being transplanted from MMD (HR 2.33,  $p 0.035$ ) and from CMV- donor with CMV+ recipient (HR 2.52,  $p 0.002$ ) were associated with higher NRM, as well as grade III-IV aGVHD (HR 4.07,  $p < 0.0001$ ). Phase CR > 1 was associated with higher risk of relapse (HR 1.95,  $p 0.001$ ) lower EFS (HR 1.73,  $p 0.001$ ) and lower OS (HR 0.54,  $p 0.001$ ), compared with CR1. Being transplanted with BM (versus other sources) and with T-depletion was protective against aGVHD. Being transplanted from MMD (HR 2.99,  $p 0.028$ ) without T-cell depletion (HR 5.65,  $p 0.009$ ) and from female donor with male recipient (HR 1.90,  $p 0.025$ ) were associated with risk of higher extensive cGVHD.

**Conclusion:** In very high risk patients, EFS and OS were significantly lower after MMD transplantation compared with MSD/MD, due to higher NRM.

**Reference:** Peters et al, *J Clin Oncol*; 2015;33: 1265-74.

**Disclosure of Interest:** None declared.

## O066

### Presence of centromeric but absence of telomeric group B KIR haplotypes in stem cell donors improves leukemia control after stem cell transplantation for childhood ALL

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**Introduction:** Allogeneic HSCT provides high cure rates for children suffering from high risk ALL. Event-free-survival (EFS) reaches 65-70% and is associated with low non-relapse mortality (NRM) (Peters, JCO 2015). Currently, results after HLA-matched unrelated donors (MUD) transplants nearly equal those using HLA-identical sibling (SIB) donors. Relapses, however, remain the major cause of treatment failure necessitating novel strategies to further improve disease control. Results from adult AML studies identified a donor KIR B haplotype being beneficial for leukemia control. This effect, however, varied depending on disease, donor type, conditioning intensity and potential graft manipulation.

**Material (or patients) and methods:** We analysed the impact of donor KIR B haplotype and chromosomal position in 209 children with ALL that were treated on the ALL-SCT-BFM-2003 trial. Results from this trial showed remission status (CR1 vs CR2) but not donor type (SIB vs MUD) or stem cell source to be a prognostic factor for outcome. Genotyping of 14 KIR genes was performed in 209 stem cell donors (65 SIB, 144 MUD) using polymerase chain reaction–sequence specific primers (Uhrberg, Immunogenetics 2002). KIR B gene content score according to Cooley (Blood, 2010), chromosomal position, and a newly developed composite KIR score incorporating KIR B haplotype presence and the respective chromosomal position were retrospectively analysed for its association with disease outcome. For statistics the Kaplan-Meier method and cumulative incidence estimates were utilized.

**Results:** The KIR B content score (B) (defined as the number of KIR B gene motifs present in the genome (range 0 to 4)) identified high score donors (7%) only (score 3/4) to be associated with a low relapse incidence ( $8 \pm 2\%$ ,  $RI \pm SD$ ) However, low scoring donors (0 to 2, 93% of donors) did not show this segregation ( $RI$  for  $B=0$ :  $22 \pm 5\%$  vs  $B=1$ :  $24 \pm 5\%$  vs  $B=2$ :  $29 \pm 7\%$ ). When comparing donors in terms of presence vs absence of a KIR B motif in the centromeric and telomeric region separately, we found  $RI$ s of  $16 \pm 4\%$  (B present) vs  $30 \pm 5\%$  (B absent) for centromeric and  $31 \pm 5\%$  (B present) vs  $18 \pm 4\%$  (B absent) for telomeric regions, respectively ( $P < 0.05$  and  $0.06$ ). Incorporating this dichotomous role (centromeric vs telomeric KIR B motif presence) into a new composite KIR B score proved to classify ALL transplant recipients with low, moderate or high  $RI$  of  $13 \pm 5\%$  vs  $21 \pm 4\%$  vs  $40 \pm 8\%$  ( $P < 0.01$ ). This translated into statistically relevant differences in EFS after transplantation when using donors of distinct composite KIR B scores:  $59 \pm 8\%$  for neutral ( $n=45$ ) vs  $73 \pm 4\%$  for better ( $n=108$ ) vs  $79 \pm 6\%$  for best donor ( $n=56$ ;  $P < 0.05$ ). Of note, the predictive power of this score even proved to be reproducible in a subgroup analysis of MUD transplants.

**Conclusion:** In contrast to recently published adult AML transplant data, we found that with regard to donor selection the combination of a missing telomeric KIR B motif with the presence of a centromeric KIR B motif results in improved leukemia control in children transplanted for ALL. Consequently, donor selection with a special focus on KIR B content and chromosomal position might prospectively reduce relapse rates substantially and warrants critical review of currently applied donor selection criteria for this group of patients.

**Disclosure of Interest:** None declared.

## O067

### The First Step to Precision Dosing of Alemtuzumab in Pediatric Allogeneic Hematopoietic Cell Transplantation Patients: Results of a Prospective Alemtuzumab Pharmacokinetic (PK) study

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**Introduction:** We recently defined an optimal target range for Day 0 alemtuzumab concentration of 0.2-0.4ug/mL that will potentially balance the risks of acute graft vs host disease (GVHD) and mixed chimerism, and maximize lymphocyte recovery following alemtuzumab, fludarabine, and melphalan reduced intensity conditioning hematopoietic cell transplantation (RIC HCT) (Marsh et al, Blood, pending). The aim of this study was to prospectively study the pharmacokinetics (PK) of alemtuzumab when given subcutaneously to pediatric patients undergoing allogeneic HCT in order to develop a population PK model to use for a Precision Dosing Trial.

**Material (or patients) and methods:** Twenty patients with non-malignant diseases receiving an alemtuzumab, fludarabine, and melphalan RIC regimen were enrolled. Seventeen patients received 1mg/kg alemtuzumab divided over 5 days subcutaneously, starting on day -14, and were included in the data analysis. The median age was 7 years (range 0.5-18 years). Blood samples were drawn for PK measurement at pre-dose, 30 minutes, and 8 hours after each dose, followed by daily levels until day +2, and weekly levels for 8 weeks. Absolute lymphocyte counts were recorded from the daily CBC results obtained as part of routine clinical care. Alemtuzumab concentrations were measured by a validated flow cytometric assay. Descriptive PK analysis was conducted using standard non-compartmental methods. The area under the plasma concentration versus time curve ( $AUC_{0-336h}$ ) was determined using the trapezoidal rule. Pre-transplant terminal half-life ( $T_{1/2}$ ) and elimination rate constant ( $ke$ ) were calculated based on concentrations at day -7 to 0 (transplant day).

**Results:** Standard PK parameters are included in Table 1. The Median alemtuzumab concentration at day 0 was 1.27ug/mL (range 0 to 2.56ug/mL). Fourteen of 17 patients had concentrations of greater than 0.4ug/mL at day 0 while 2 patients showed concentrations within the predefined target range of 0.2-0.4ug/mL. Day 0 levels correlated negatively with pre-dose ALC (data not shown). Some correlation of Day 0 levels with underlying diagnosis was also observed, as a trend towards decreased levels in patients with HLH was observed ( $P=0.07$ ). The median terminal  $t_{1/2}$  was 5.2 days. Day 0 concentrations correlated positively with concentrations at Day -7 and AUC, and inversely with alemtuzumab elimination rate (data not shown).

Table 1. Alemtuzumab PK parameter estimates.

Parameter	Median (25-75th percentiles)
Maximum concentration (µg/mL)	2.39 (1.97-3.25)
Concentration at Day 0 (µg/mL)	1.27 (0.33 – 1.53)
$AUC_{0-336h}$ (hr*mg/L)	412 (343 – 650)

**Conclusion:** Here we report the PK of subcutaneous alemtuzumab given to pediatric allogeneic HCT patients. Almost all patients had persistence of lytic levels of alemtuzumab beyond day 0, at levels which are in excess of that needed to reduce the risk of acute GVHD. Levels correlated with pre-transplant ALC, and also diagnosis. These results will allow the development of a Precision Dosing Algorithm to attain optimal alemtuzumab levels at Day 0 in order to balance the risks of acute GVHD and mixed chimerism and maximize lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT.

**Disclosure of Interest:** None declared.

O068

**The Minimum Required Level of Donor Chimerism in Hereditary Hemophagocytic Lymphohistiocytosis – A Retrospective Study with 103 Patients**

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**Introduction:** Hereditary hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome which can only be cured by hematopoietic stem cell transplantation (HSCT). Reduced-intensity conditioning regimens have improved survival after HSCT for HLH, but have increased the frequency of mixed chimerism. In mice, a proportion of 10-20% donor chimerism (DC) prevents HLH reactivation. The minimum protective level of DC in humans remains to be determined.

**Material (or patients) and methods:** Patients transplanted for hereditary HLH between 2000 and 2013 with a DC < 75% at least once (overall and/or CD3<sup>+</sup> and/or CD56<sup>+</sup> subpopulations) were included in this retrospective multicenter study. Data on DC was correlated with specific immunologic function, occurrence of systemic reactivations ( $\geq 5/8$  HLH criteria), partial flares (< 5 criteria and HLH-directed treatment), isolated CNS reactivations, and management.

**Results:** 103 Patients from 23 hospitals in 7 countries were enrolled. A reactivation occurred in 10, a partial flare in 3 and an isolated CNS HLH in 4 patients. Ten events occurred during the phase of profound immune suppression until day+180 at a median DC of 10%, range 1-100% (CD3<sup>+</sup> if available, otherwise overall), which renders a differentiation between secondary post-HSCT HLH and HLH related to the genetic defect difficult. Seven recurrences developed between 6 months and 6.7 years post HSCT (median DC 10%, range 0-30%). Fourteen patients had a DC  $\leq 30\%$  for more than 6 months without reactivation. In 5 of them, overall and lineage-specific DC were even  $\leq 10\%$  for a median of 5.1 years (range 1.1–10). The proportion of severe genetic defects (defined as HLH onset during infancy) was similar in patients with recurrence as compared to patients with long-term low-level DC  $\leq 30\%$  without recurrence (59% and 64%, respectively). The outcome of patients with a DC nadir  $\leq 30\%$  was significantly inferior compared to patients where the DC level never fell below that level. The hazard ratio of overall and event-free (event defined as HLH recurrence, 2<sup>nd</sup> HSCT, or death) survivals were 3.5 and 4.6, respectively. A 2<sup>nd</sup> HSCT was performed in 18 patients (median overall DC of 4%; range 0-19%). Death due to reactivations occurred in 4 patients (24% of recurrences), in comparison to 6 patients who died after complications related to the 2<sup>nd</sup> HSCT (33% of 2<sup>nd</sup> HSCT).

**Conclusion:** Beyond the phase of profound immune suppression, DC > 20-30% usually protects against reactivations, lower levels however do not inevitably result in recurrences. A threshold for the early phase with profound immune suppression cannot be reliably determined. The risks of a

pre-emptive 2<sup>nd</sup> HSCT must be weighed against the risk of reactivation.

**Disclosure of Interest:** None declared.

O069

**Combining Clofarabine/fludarabine with Exposure Targeted Busulfan for Pediatric Leukemia: An Effective, low Toxic TBI-free Conditioning Regimen**

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**Introduction:** The combination of Clofarabine + Fludarabine + Busulfan (CloFluBu) was found to have synergistic anti-leukemic activity against ALL and AML blasts *in vitro* (Andersson et al: BBMT 2011). As TBI induces significant late effects in childhood ALL, and AML patients have high relapse rates, we hypothesized that CloFluBu may be an interesting alternative to TBI in ALL and add anti-leukemic activity in AML. Within the "Dutch COG HCT Working Group" we prospectively studied the outcomes of CloFluBu-conditioning regimen for lymphoblastic and myeloid malignancies.

**Material (or patients) and methods:** Patients from the 2 pediatric HCT programs (LUMC and UMC Utrecht) in the Netherlands with a lymphoblastic or myeloid malignancy receiving their first HCT were included from Aug-2011 to Aug-2015. Clofarabine 30mg/m<sup>2</sup> was given in 1 hour, followed by Fludarabine 10mg/m<sup>2</sup> in 1 hour followed by a 3-hour infusion of once-daily targeted busulfan (weight-based dosing + therapeutic drug monitoring to a Bu-exposure of 90mg\*h/L over 4 days). Thymoglobulin was added in unrelated donors (except in AML receiving cord blood: CB). GvHD-prophylaxis was according to standard protocols. Primary endpoint: Leukemia free survival (LFS). Other endpoints: acute- and chronic graft-versus-host disease (GvHD), transplantation related mortality (TRM), VOD/SOS, non-infectious lung injury. A predictor analysis was performed using Cox Proportional Hazard Models.

**Results:** Eighty-seven patients were included: 37 AML (31 CR2, 6 CR1), 21 ALL-CR1 (incl. 2Ph+), 4 Infant-ALL-CR1, 15 ALL-CR2/3, 10 other (6 MDS, 3 CML, 1CNL; chronic neutrophile leukemia). In 29 ALL patients MRD status prior to HCT was available: 14 Neg, 7 > 10e-3 (high) and 8 < 10e-3 (low). Donors used: 35 unrelated CB (6/6; 17, 4-5/6; 18), 20 matched Family donors (FD), 2 mismatched FD, and 30 MUD (10/10; 21, 8-9/10; 9). Median age at HCT: 10.9 (0.5-18.8) years. Median follow-up 455 (range 18-1665) days. The estimated 2-year LFS was 75+/-5% (AML 76+8%, ALL-CR1 95+5%, other 87+11%, ALL-CR2-3 56+13%), with an estimated TRM @ 1year of 4,3 +/-3% and relapse @ 2year 21+/-5%. Seven ALL-patients had previous CNS disease (at diagnosis or relapse). Of the 7 ALL relapses after HCT 1 isolated CNS relapse was noted and 2 combined BM+CNS relapses (2 had previous CNS-disease). All (n=4) MRD+(high) patients in ALL-CR2/3 relapsed after HCT. In ALL-CR1 no influence of MRD-status on relapse (3MRD-high, 4MRD-low). Other endpoints: 2 graft-failure were noted (2.5%: 1 MUD successfully re-grafted with CB, 1 mFD), aGvHD 2-4 @ day 180 was 25+/-5% (grade 3-4: 12+4%), extensive cGvHD @ 2year 7+3.5%, non-infectious lung-injury @ 2-years 10+4% and no VOD/SOS (0%) was noted.

**Conclusion:** CloFluBu in myeloid- and lymphoblastic malignancies, showed very limited toxicity and encouraging LFS in all groups. A longer follow-up in a larger cohort is needed to draw firm conclusions with regards to the anti-leukemic effect and late effects.

**Disclosure of Interest:** None declared.

## O070

### Fertility preservation issues in pediatric transplantation. Work in progress on a consensus by the EBMT PD WP Fertility Panel

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**Introduction:** Current approaches to fertility preservation vary between countries due to regulatory, scientific, financial and cultural issues. Increased awareness and recent advances in reproductive medicine prompted a panel of fertility specialists within the EBMT PD WP to produce guidelines.

**Material (or patients) and methods:** A trained team should be available in all transplant centers for counseling children/adolescents and/or their families. Information should be provided on: 1. infertility risk (previous chemotherapy, conditioning, TBI, alkylating agents); 2. available preservation techniques, which, other than sperm preservation, may be considered experimental in prepubertal children; 4. specific

timeline for each technique, which may not be compatible with the treatment schedule needed to control the disease; 6. patient performance scores and conditions (neutropenia, thrombocytopenia, infection) may limit surgery feasibility. 2. informed consents detailing procedures, associated risks and costs must be signed by patients and/or parents.

**Results:** Interventions should be tailored according to pubertal development (Tanner stage, testis size, menarche, erection, ejaculation) and patient/parent motivation

**Boys**  
Sperm collection and cryopreservation in postpubertal boys: 1. it carries no risk and should be mandatory; 2. it should be ideally organized at diagnosis in malignant diseases, before chemotherapy causes virtually invariable azoospermia; 3. appropriate samples may be collected at least two months after chemotherapy discontinuation; 3. young post-pubertal boys unable to ejaculate may be offered electric stimulation under sedation;

Testicular tissue biopsy and cryopreservation in prepubertal boys: 1. require special cryopreservation techniques; 3. highly experimental: no conceptions reported to date with this approach.

#### Girls

Ovarian hormonal hyperstimulation and transvaginal oocyte collection and cryopreservation in postpubertal girls: 1. established technique in adult females, 2. transvaginal access may be contraindicated in pre-coitus girls; 3. it should be ideally organized as soon as eligibility for transplant is clear, before chemotherapy causes germ cell loss; 4. thrombotic risk associated with hormonal therapy should be evaluated;

Ovarian tissue laparotomic collection and cryopreservation: 1. only feasible approach in prepubertal girls, also feasible postpuberty; 2. whole prepubertal and at least half post pubertal ovary should be removed (ovarian function reduction per se must be considered); at present healthy children have been born after re-implantation of adult ovarian tissue; prepubertal ovarian tissue maturation is experimental; GnRH agonists during chemotherapy may reduce risks of premature ovarian failure in post pubertal girls.

General comments on gonadal collection: 1. only feasible approach in prepubertal children, also feasible postpuberty; 2. highest contamination risk in leukemia among malignancies; collection may be considered for *in vitro* fertilization; 4. gonadal tissue should be reserved for reproductive purposes; hormonal replacement therapy per se may not justify gonadal removal/implantation. 5. cryopreservation should be considered for any gonadal tissue removed for diagnostic or therapeutic purposes.

**Conclusion:** This consensus will be available to transplant centers and health authorities for regulatory planning to support fertility preservation projects.

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**O071**

**Multicentre study of a treosulfan-based conditioning regimen for children with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) undergoing allogeneic haematopoietic cell transplantation (allo-HCT)**

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**Introduction:** Allogeneic haematopoietic cell transplantation (allo-HCT) is a potential cure for children with AML and MDS. Traditional full myeloablative regimens containing busulfan are the most commonly used for children with AML/MDS. These regimens are effective, but are limited in part by toxicity and transplant-related mortality (TRM). Treosulfan is a bifunctional alkylator recently evaluated in HCT regimens for children and adults, with the goal of reducing toxicity and TRM. We present the results of a multicentre, prospective phase II study evaluating the safety, preliminary efficacy and pharmacokinetic (PK) profile of a Treosulfan-based regimen in children with AML or MDS undergoing allo-HCT.

**Material (or patients) and methods:** Forty patients were enrolled at 13 centres in the United States from Sep 2013 to Apr 2014. Median age was 11 years (1-19). Patients were transplanted for AML (*n*=32) in first (*n*=18), second (*n*=11), third or greater CR (*n*=3), or MDS (*n*=8). Stem cell sources included bone marrow (BM, *n*=25), peripheral blood stem cells (PBSC, *n*=6), or cord blood (CB, *n*=9). Of the 31 PBSC/BM grafts, 10 were from HLA-matched relatives and 21 from unrelated donors. All CB donors were unrelated, 2 recipients of double CB grafts. The regimen consisted of Treosulfan IV at a dose of 10, 12, or 14 g/m<sup>2</sup>/day (based on BSA of ≤ 0.5, > 0.5 – 1.0 and > 1.0 m<sup>2</sup>, respectively) on days -6 to -4, Fludarabine (30 mg/m<sup>2</sup>/day) IV on days -6 to -2 and 200 cGy TBI on day -1. Graft-versus-host disease (GVHD) prophylaxis for those receiving BM or PBSC was with tacrolimus/methotrexate, and cyclosporine/mycophenolate for CB. Serial blood samples (1 mL) were collected for 19 patients weighing < 40 kg for PK studies prior to and after the treosulfan infusion (0, 20, 40 min, and 1,2,3,4,5 & 24 hours post infusion). Samples were batched and analysed by high-performance liquid chromatography with refractometrical detection. Parameters analysed included area under the curve (AUC), maximum concentration (Cmax), half life (t<sub>1/2</sub>), volume of distribution (Vss) and total clearance (Cl).

**Results:** One year overall (82%) and disease-free survival (72%) are excellent and similar to those expected with conventional myeloablative regimens in children. The 1-year relapse rate was 25%, 28% for AML and 13% for MDS. Cumulative

Table 1. PK profile of treosulfan in children with AML/MDS:

Parameter	10 g/m <sup>2</sup>	12 g/m <sup>2</sup>	14 g/m <sup>2</sup>
N	5	10	4
Age, yrs **#	1 (0.9-1)	6 (4-8)	9 (9-11)
BSA, m <sup>2</sup> **#	0.43 (0.38-0.50)	0.86 (0.52-0.99)	1.11 (1.05-1.40)
Weight, kg **#	9.2 (8.1-14.6)	22.7 (19.4-25.1)	30.7 (30.1-37.5)
AUC, mcg/mL·h	2762 ± 1058	2240 ± 538	2235 ± 96
Cmax, mcg/mL	977 ± 412	799 ± 201	788 ± 28
Terminal, t <sub>1/2</sub> (h)	1.39 ± 0.25	1.49 ± 0.14	1.38 ± 0.11
Vss, L **+	6.7 ± 2.3	9.5 ± 2.5	9.9 ± 0.6
Cl total (mL/min) **+	69.4 ± 22.5	94.9 ± 18.2	104.5 ± 4.5

Age, BSA and weight expressed in median (range); AUC, Cmax, t<sub>1/2</sub>, VSS and CL expressed in mean ± SD.

\*\*# = significant differences (p < 0.05) between 10 vs. 12 g/m<sup>2</sup>, 10 vs. 14 g/m<sup>2</sup>, and 12 vs. 14 g/m<sup>2</sup>, respectively using t-test.

incidence of TRM at one year was remarkably low at 3%, with one death due to GVHD. All other deaths (*n*=7) were from relapse. No serious organ toxicities were observed, including sinusoidal obstruction syndrome. Trilineage engraftment was seen in all patients. Cumulative incidences of grade II-IV and III-IV acute GVHD by day 100 were 19% and 9%, respectively. Chronic GVHD developed in 31% of patients by 1 year. BSA-based treosulfan dosing reliably resulted in predictable AUC and Cmax, which is required for dosing without measuring PK (Table 1). Though there were significant differences observed in Cl and Vss across treosulfan dose groups, these differences did not seem to impact disease control or regimen toxicity.

**Conclusion:** A conditioning regimen using a BSA-based treosulfan dose results in excellent engraftment and disease free-survival, minimal toxicity and TRM in children with AML/MDS, with similar outcomes for children of different size.

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O072

**Risk factors in stage 4 neuroblastoma patients treated with Busulphan-Melphalan. Report from the European High Risk Neuroblastoma HR-NBL1/SIOPEN TRIAL**

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**Introduction:** Evaluation of the risk factors in high risk metastatic neuroblastoma (NBL) patients (pts) treated with busulfan-melphalan (BUMEL) in the HR-NBL1/SIOPEN trial.

**Material (or patients) and methods:** Since 02/02/2002, 901 patients received BUMEL and were accrued in 20 countries (149 centres). All stage 4 pts > 1year (yr) at diagnosis up to 21 yrs were eligible whereas pts < 1year only in the presence of MYCN amplification (MNA). After Rapid Cojec or modified N7 induction, pts with insufficient response received additional 2<sup>nd</sup> line treatments (i.e. TVD 2- 4 courses) to proceed to BUMEL myeloablative therapy. Local control aimed at complete surgical resection (achieved in 76%) and radiotherapy of 21 Gy was given only to the primary tumour site. Till 2007 maintenance treatment was 13cis RA alone. In 2007 ch14.18/CHO mAb based immunotherapy (IT) was introduced. The median age at diagnosis is 3.0yrs (range, 1month-19yrs). At diagnosis 127pts were ≤ 1.5 yrs, 600 between 1.5 to 5 yrs and 174 > 5yrs. MNA was present in 340pts. Additional TVD was reported for 254 patient. Intravenous busulfan was used in 600pts. Response prior to BUMEL was CR in 282pts (24% with TVD), VGPR in 322pts (31% with TVD), PR in 224 pts (29% with TVD) whereas response was not specified in 73 pts (27 % had TVD). A total of 352 patients received ch14.18/CHO based IT. **Results:** Severe toxicity rates (ICU, toxic deaths) are 7%, the VOD rate is 24% (grade 3: 4%).

Outcomes are reported here as 5-yrs EFS rates. Age has a major influence: EFS was 0.59 ± 0.09 for age < 1yr, 0.55 ± 0.06 for 1- 1.5yrs, 0.38 ± 0.02 for 1.5yrs-5yrs and > 5yrs 0.22 ± 0.04

(P=0.006). There was no significant difference in outcome for patients with or without TVD courses shown by EFS of 0.33 ± 0.04 in 254 pts and 0.39 ± 0.02 for 647 pts. Patients in CR prior to BUMEL had a significantly better outcome with an EFS of 0.45 ± 0.03 as opposed to 0.35 ± 0.03 for VGPR, 0.34 ± 0.04 for PR and 0.30 ± 0.07 in those with unspecified response (P=0.001). Patients with one isolated metastatic site (EFS 0.53 ± 0.06) fair significantly better than those with multiple sites (EFS 0.36 ± 0.02) (P=0.008). COX regression in stage 4 patients > 1yrs confirms combined metastatic sites and CR& VGPR as independent factors but also shows a significant interaction for age and MNA with a significant age effect only in pts without MNA. In stage 4 pts without MNA age 1-1.5yrs (25 pts) the EFS was 0.96 ± 0.04, whereas it was 0.33 ± 0.03 for 1.5- ≤ 5yrs and 0.25 ± 0.05 for > 5yrs. However EFS was 0.59 ± 0.09 in MNA infants.

**Conclusion:** Younger age, an isolated metastatic site only and response better than PR prior BUMEL are major prognostic factors. MNA infants clearly benefit from intensification with BUMEL.

**Disclosure of Interest:** None declared.

O073

**Hematopoietic Stem Cell Transplantation from HLA Identical Sibling For Sickle Cell Disease: an International Survey on Behalf of Eurocord-monacord, Ebmt Paediatric Disease Working Party and CIBMTR**

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**Introduction:** Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for sickle cell disease (SCD). However, HSCT is offered to relatively few patients (pts) with SCD for reasons including lack of a suitable HLA-matched donor, lack of consensus on indications for HSCT, the potential for trading one chronic condition for another, such as chronic

graft-versus-host disease (GVHD), and the mortality associated with the procedure.

**Material (or patients) and methods:** We report outcomes after HLA-matched sibling HSCT of 1,000 pts with SCD transplanted in EBMT or CIBMTR centers. HSCT were performed between 1986 and 2013;  $n=439$  from CIBMTR and  $n=561$  from EBMT centers.

**Results:** Median age at HSCT was 9 years (1-54y); 85% of pts were aged <16 years and 94% were homozygotes for hemoglobin S. The most common indication for HSCT was stroke. Other indications included: central nervous system event lasting longer than 24 hours, elevated cerebral arterial velocity, acute chest syndrome and vaso-occlusive crisis requiring hospitalization. Most HSCTs (87%) used myeloablative-conditioning regimens. Most pts received *in vivo* T-cell depletion (71%). The predominant GVHD prophylaxis was cyclosporine+methotrexate (56%). The cell sources for HSCT were bone marrow, BM (84%), cord blood, CB (9%) and peripheral blood, PB (7%). The median follow-up was 45 (1.1-324.6) months. The cumulative incidence function (CIF) of neutrophil engraftment at day+60 was 98% (96.6% for CB, 98.3% for BM and 95.2% for PB). The CIF of platelet engraftment was 98% ( $96 \pm 2\%$  for CB,  $99 \pm 1\%$  for BM and  $98 \pm 9\%$  for PBSC). Forty-five pts experienced secondary graft failure; 67 pts died mainly due to GVH or infection. The 3-year overall (OS) and event-free survival (EFS) were 94% and 90%, respectively. According to stem cell source, 3-year OS was 99% after CB, 94% after BM and 80% after PBS ( $P < 0.0001$ ). In multivariate analysis, every year in age increment (HR 1.1, 95%CI 1.07-1.14,  $P < 0.001$ ) and use of PB (HR 3.43, 95% CI 1.49-7.88,  $P = 0.004$ ) was associated with higher mortality. EFS was lower with every year in age increment (HR 1.08, 95%CI 1.04-1.1,  $P < 0.001$ ), PB grafts (HR 2.03, 95% CI 1.01-4.08,  $P = 0.48$ ). CI of acute GVHD grade 2-4 was 14.4% (12.2-16.7) of chronic GVH 13.3% (11-15.8). Risks of acute GVHD were higher with increasing age (HR 1.04 95%CI 1.01-1.07,  $P = 0.008$ ). None of the variables tested were associated with chronic GVHD.

**Conclusion:** This large registry based international study shows that HLA identical sibling transplant is successful in more than 90% of the pts with severe SCD with limited transplant related complications (rejection, GVHD). Strategies aimed at lowering graft failure and GVHD are desirable to further optimize the observed 3-year event-free survival. Importantly, these data should increase awareness to early referral to HSCT of pts with severe SCD.

**Disclosure of Interest:** None declared.

#### O074

##### Excellent results after RIC-conditioning and T-cell replete HLA-identical transplants including cord blood in hemophagocytic lymphohistiocytosis (HLH)

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**Introduction:** Reduced toxicity conditioning regimens containing submyeloablative iv. busulfan (cum. AUC 45-65 mg/Lxh), high-dose fludarabine 180 mg/sqm (d-8 to -3; in infants <9 kg 6x1.2 mg/kg), serotherapy (rabbit ATG or low-dose alemtuzumab) and GVHD-prophylaxis comprising CSA (d-3 to d+160) and MMF (d±0 to +80-100) have shown excellent results in high-risk chronic granulomatous disease patients. Its efficacy in other non-malignant diseases, e.g. HLH, is still unknown.

**Material (or patients) and methods:** In this present study (6/2009 to 6/2015), we analyzed the outcome after administration of this protocol in children and AYA with hemophagocytic lymphohistiocytosis (age: med 0.83; range 0.27-21 yrs):  $n=3$  with MUNC 18-2,  $n=5$  with MUNC-13-4,  $n=2$  with XLP,  $n=1$  CHS and  $n=2$  with HLH with unknown genetic cause but abnormal degranulation assays.

**Results:** Transplants comprised  $n=9$  unrelated ( $n=7$  HLA-10/10 and  $n=2$  HLA-9/10) and  $n=4$  related HLA-identical donors. The donor sources were  $n=7$  BM,  $n=4$  PBSC and  $n=2$  CB. Intravenous total busulfan doses ranged between 4.4 and 17.2 mg/kg (median 12 mg/kg) corresponding to cumulative AUC of 48-77 mg/Lxh (median 63.5). Fludarabine was administered at a total dose 180 mg/sqm ( $n=10$ ) or  $6 \times 1.2$  mg/kg ( $n=3$ ) depending on body weight. Serotherapy comprised alemtuzumab ( $n=9$ ; range 0.8 - 2.5, median 0.6 mg/kg) and ATG ( $n=1$ , ATG-Fresenius, 40 mg/kg;  $n=3$ , Thymoglobuline 7.5 mg/kg).

All patients engrafted. No graft failures, 2 cases of reversible VOD and one case of reversible pulmonary hypertension was observed. After a median follow-up of 34 months (range 4-65 mo), the overall and event free survival rates are 100/100%, respectively. The rates of acute GVHD III-IV and of cGHVD were 0/0%, respectively, at last follow-up. All surviving patients exhibit a stable myeloid donor chimerism ( $n=3$  80-90%;  $n=9 >95\%$ ).

**Conclusion:** Submyeloablative targeted busulfan, high-dose fludarabine and serotherapy containing RIC-regimens are excellent treatment options in HLH-patients with T-cell replete HLA-identical transplants including unrelated cords.

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**Disclosure of Interest:** None declared.

#### O075

##### The changing patterns of graft failure in MPSIH, Hurler syndrome: A review of 30 years experience

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**Introduction:** Haematopoietic stem cell transplant (HSCT) is the standard of care in children with Hurler syndrome as it is the only curative therapy that stops or reverses the disease progression. One of the major challenges in HSCT for Hurler syndrome is graft failure. Here, we examined the evolving pattern of graft failure over the past three decades.

**Material (or patients) and methods:** Retrospective review of all HSCT done in children with Hurler syndrome at Blood and Bone marrow transplant unit, Royal Manchester Children's Hospital over a thirty-one-year period (1985 - 2015). A full-intensity Busulfan-based conditioning regimen with pharmacokinetic-guided dosing (Bu-pk) was started from September 2004. We compared outcomes and patterns of graft failure before and after this change in 2004. Since 2004, Bu/Cyclo was used from September 2004 to June 2010 and Bu/Flu from July 2010 to December 2015. The conditioning

regimens used before 2004 were variable. Serotherapy used was either alemtuzumab or ATG.

**Results:** 119 transplants were performed in 97 patients. The median age at first HSCT was 13.4 months (range 3.9 – 66 months; the median age in pre-2004 was 14.3 months and post-2004 was 12.4 months ( $P=0.154$ )). The graft source was marrow ( $n=49$ , 50.5%), peripheral blood ( $n=15$ , 15.5) and cord blood ( $n=33$ , 34%). The 5-year overall survival improved from 66.7% to 90.2% while the 5-year event-free survival increased from 30.6% to 83.6% in modern era. The incidence of graft failure reduced significantly from 47.2% (17/36 patients) pre-2004 to 8.1% (5/61 patients) in the modern era. Of the 17 patients (21.9%) with graft failure pre 2004, most ( $n=14$ ) had late graft failure with autologous reconstitution and 3 had primary aplasia. In the modern era, 30 patients (49%) received cord blood transplant and the remaining 31 patients (51%) received either marrow ( $n=20$ , 32.7%) or peripheral blood ( $n=13$ , 21.3%). All the 5 patients with graft failure received 6/6 cord blood transplant. 3 had primary aplasia whereas 2 had secondary aplasia after they had achieved prior donor-derived engraftment with red cell and platelet transfusion independence. Predictors for graft failure were analysed. All 5 patients received second transplants (2 second CB; 2 marrow; 1 PB). 4 were alive and engrafted while one died of adenovirus pneumonitis.

**Conclusion:** The pattern of graft failure has changed from primarily autologous reconstitution in pre-Bu pK era to marrow aplasia (both primary engraftment failure and a unique, late, immune-mediated aplasia after FluBu) following cord HSCT in the modern era.

**Disclosure of Interest:** None declared.

#### O076

##### **Haploidentical Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Patients with X-linked Adrenoleukodystrophy: expanding the donor pool in an urgent situation**

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**Introduction:** X-linked adrenoleukodystrophy (X-ALD) is a disorder caused by a defect in the metabolism of very long chain fatty acids leading to demyelination and neurodegeneration. The most severe form of X-ALD is the cerebral variant, which typically leads to severe disability and death during the first two decades. To date, allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment that has shown to significantly change the natural history of the disease, enhancing survival and stabilizing neurologic lesions and symptoms. Only 30% of patients who might benefit from HSCT will have a full HLA-matched donor. In these urgent situations, haploidentical family members might be an option for these rapidly progressing diseases. Haploidentical HSCT using post-transplant cyclophosphamide has been performed in series of malignant and non-malignant diseases and has shown similar outcomes compared to other alternative donor sources. Here we show our experience with 9 patients with X-ALD treated with haploidentical HSCT with post-transplant cyclophosphamide.

**Material (or patients) and methods:** Between november 2012 and august 2014, 9 patients with X-ALD (ages 6 to 18 years) underwent 12 related haploidentical HSCT in two different institutions, three patients received second transplants with different related donors. One patient received a second transplant after failure of a double cord blood transplant. Pre-transplant MRI showed Loes score of 2,5 to 18, all patients had neuropsychological evaluation with performance IQ above 90. Donor was the father ( $n=8$ ), the

uncle ( $n=3$ ) or brother ( $n=1$ ). Eight patients received reduced toxicity conditioning regimen consisted of: fludarabine 30 mg/m<sup>2</sup> on days -6 to -2, cyclophosphamide 14.5 mg/kg on days -6 and -5, and total body irradiation 2 Gy on day -1. Six patients received also rabbit antithymocyte globulin 0.5 mg/kg on day -9 and 2 mg/kg on days -8 and -7. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg/d on days +3 and +4, tacrolimus or cyclosporine A and mycophenolate mofetil starting on day +5.

**Results:** Seven patients engrafted, 13 to 18 days after transplant. One patient had a primary graft failure and was not eligible for a second transplant due to severe progression of neurological symptoms. Three patients had secondary graft failure with progressive loss of donor chimerism and were successfully rescued with second haploidentical transplants using different related donors. One patient developed late graft failure and is waiting for a second transplant. After this initial disappointing experience with 5 out of 8 patients developing graft failure we changed the conditioning regimen and a patient received same Fludarabine and Cyclophosphamide regimen associated with 4Gy total lymphoid irradiation. Eight patients are alive and engrafted from 20 to 33 months after first and second transplants, with chimerism from 80 to 100% donor cells. Seven patients had neurologic progression of symptoms reflecting either advanced disease at transplant or progression after HSCT.

**Conclusion:** In conclusion, haploidentical HSCT with post-transplant cyclophosphamide is a feasible alternative for X-ALD lacking a suitable matched donor. Graft failure is still an obstacle that has to be better prevented.

**Disclosure of Interest:** None declared.

#### O077

##### **Molecular basis, therapy, complications and follow-up in a cohort of patients affected by Wiskott-Aldrich Syndrome: the experience of the Children Hospital of Brescia**

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**Introduction:** Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency, characterized by microthrombocytopenia, recurrent infections, eczema and high risk for the development of malignancy and autoimmune diseases. It is caused by mutation in the WASP gene, which effects on the expression of the WASP protein, a hematopoietic cytoskeletal protein. The only curative therapy for WAS is hematopoietic stem cell transplantation (HSCT), which has to be performed at an early age, while gene therapy is under study.

**Material (or patients) and methods:** We retrospectively analyzed our experience with 39 WAS patients followed from 1990 to 2015 at the Children Hospital of Brescia, Italy. All the patients presented, at diagnosis, microthrombocytopenia, clinic features valuated with Zhu's score and mutation of the WASP gene. In 32 patients we evaluated the expression of WASP protein on blood cells, which was absent or reduced. In all patients diagnosis was confirmed by molecular analysis. 37 patients underwent HSCT, of whom 6 patients from a related identical donor (RID), 3 from a mismatched related donor (MMRD) and 28 from matched related donor (MUD).

**Results:** Overall survival is 72% (27/37). All the RID BMT (6/6) and 80% (22/28) of MUD BMT recipients survived, while the 3 MMRD BMT recipients died. Deaths are related to severe infections and bleedings. All the RID BMT recipients are fine and well, while among the MUD BMT recipients survived, 58% (10/17) presented symptoms related to microthrombocytopenia, autoimmune hypothyroidism and skin's affections. Hematopoietic stem cells engraftment was present in 95% of the patients. In some cases we observed a reduction of donor chimerism but we noticed that at least 30% can guarantee



platelets production. Mean platelets count after BMT was 237.880 mm<sup>3</sup>.

**Conclusion:** HSCT remains the gold standard therapy for WAS, although we should make more effort in reducing its complications and late effects.

**Reference:** "Current and emerging treatment options for a Wiskott-aldrich syndrome", Worth AJ et al. 2015; 11 (9), 1015-32, Expert.Rev. Clin. Immunol.

**Disclosure of Interest:** None declared.

#### O078

##### **Hematopoietic stem cell transplantations in Wiscott-Aldrich syndrome patients from haploidentical and matched unrelated donors with TCR alpha/beta and CD19 depletion of the graft – a single-center experience**

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**Introduction:** Since the first transplantation in a patient with Wiscott-Aldrich in 1968, HSCT has become a routine curative therapy for WAS, with progressively improving outcomes. TCRab/CD19 depletion is a new method of graft manipulation aimed at reduction of GVHD in allogeneic HSCT. We present our experience with TCRab/CD19 depleted HSCT in both haploidentical and unrelated donor transplants in patients with WAS.

**Material (or patients) and methods:** Between 2012 -2015 twelve WAS patients were transplanted with TCRab/CD19 depleted grafts in our center. Median age at HSCT was 1.6 years (0,9-6,6). Ten patients received transplants from unrelated donors (1 - 8/10; 3 - 9/10; 6 - 10/10 HLA matched) and two - from haploidentical donors (mothers). Eleven received Fludarabine 150mg/m<sup>2</sup>/Treosulfan 36-42g/m<sup>2</sup>-based conditioning, one additionally received Melphalan 140mg/m<sup>2</sup>. Eleven received serotherapy, one patient with severe preexisting CMV infection received only cyclophosphamide 100 mg/kg. All received calcineurin inhibitor for GVHD prophylaxis, nine also received a short course of methotrexate. Average dose of infused CD34+ cells was 11,9x10<sup>6</sup>/kg (8,8-21,3), TCRab cells 12,3x10<sup>3</sup>/kg (3,4-60,5).

**Results:** Engraftment has been achieved in all patients; median neutrophil engraftment time was 17 days (13-28), median platelet engraftment time – 13 days (7-17). Cumulative incidence of acute GVHD grade 2 was 0,33, all cases responded to steroids, none of the patients developed aGVHD grade 3-4. The incidence of chronic GVHD was 0,28. Cumulative incidence of CMV reactivation was 0,58. Frequency of CMV disease was 33,3% (three patients – CMV retinitis, one – CMV encephalitis, retinitis). One patient with severe pretransplant CMV infection with high viremia and retinitis was excluded from the analysis of CMV disease. There were no cases of PTLD. The median follow up - 18,2 months (1,2-37). Overall survival after HSCT was 0,92, one patient died of acute ADV hepatitis, the cumulative TRM was 0,08. Event-free survival (graft rejection, death were counted as events) was 0,75. Two patients (after MUD HSCT) rejected grafts 2,3 and 2,7 months post-HSCT, respectively. The incidence of rejection was 0,17. Both patients are alive after retransplantations.

Seven patients had >90% donor chimerism at last follow up. Three had persistent mixed chimerism (27-80% donor), predominantly in myeloid lineage, but had normal or moderately decreased platelet and neutrophil counts. One patient required donor lymphocyte infusions and another one received boost infusion of stem cells.

All patients had good (74-99% donor) CD3+ chimerism with adequate kinetics of the immune recovery.

**Conclusion:** TCRab/CD19 depletion is a promising method of graft manipulation for unrelated and haploidentical HSCT in

patients with WAS. Our small series demonstrate low incidence of severe GVHD and viral reactivations. The problem of high incidence of rejection and mixed chimerism may be resolved by addition of a second alkylating agent to the conditioning regimen.

**Disclosure of Interest:** None declared.

#### O079

##### **Trial comparing HSCT vs transfusions in sickle cell anemia (SCA)-patients with abnormal cerebral velocities : cerebral vasculopathy outcome at 1 year**

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**Introduction:** « Drepagrefe » is a French national prospective trial (AP-HP, NCT 01340404) involving SCA-children, younger than 15 years, placed on long-term transfusion program (TP) because of a history of abnormal-TCD (TAMMX  $\geq$  200 cm/sec), and comparing for the first time the outcome of cerebral vasculopathy following TP or HSCT. The 2 arms were defined by the random availability of a genoidentical donor. Patients with at least one non-SCA sibling, and parents accepting HLA typing and HSCT if a genoidentical donor was available were included ( $n=67$ )

**Material (or patients) and methods:** 7/67 patients had a history of stroke. Transplanted patients ( $n=32$ ) received a conditioning regimen of Busilvex, CY200 mg/kg and 20 mg/kg rabbit-ATG with CSA and a short course of MTX or MMF for GvHD prophylaxis. In the TP arm ( $n=35$ ), HbS% was maintained at < 30%, with an Hb at 9-11g/dL. At enrollment and 1 year post enrollment, blood screening, Doppler, and cerebral MRI/MRA were performed along with cognitive performance testing, the latter being done in parallel in the non-SCA siblings. Preliminary findings on cerebral velocities as the primary endpoint were reported at the last EBMT meeting and demonstrated that all patients were alive and that the 32 transplanted patients had no chronic GVHD and the same hemoglobin profile as their donor. Velocities were significantly lower post-HSCT than under TP ( $P < 0.001$ ), and were normalized in a greater number of patients ( $P=0.005$ ). We report here the cerebral imaging and cognitive performance data performed at enrollment and 1 year. A scoring system was used for classify ischemic lesions, atrophy on MRI and stenoses and Moya on MRA. Cognitive testing using the WPPSI-3, WISC-4 or WAIS-3 scales, depending on the age, was performed in patients and in siblings when possible.

**Results:** MRI/MRA data were available in 66/67 patients. At enrollment (M0), ischemic lesions and stenoses were present in 25 and 35/66 patients, respectively. Cognitive testing was obtained in 64 patients and 56 siblings. Paired analysis with siblings showed significant differences in Verbal Comprehension Index (VCI) with a mean difference of  $7.6 \pm 14.5$  ( $P < 0.001$ ), Processing Speed Index (PSI)  $6.3 \pm 20.5$  ( $P=0.04$ ), and Full Scale IQ (FSIQ)  $7.3 \pm 15.0$  ( $P=0.01$ ). After exclusion of the 7 patients with stroke history, significant differences were still observed in VCI ( $P=0.013$ ) and FSIQ ( $P=0.019$ ). Patient cognitive performance indexes were correlated negatively and significantly with the MRI and MRA scores. At 1 year, one new patient developed ischemic lesions in the TP arm but none in the HSCT arm. MRI/MRA scores were not different at 1 year between 2 arms. However a significant improvement in the MRA score was observed post-HSCT but only in no-stroke patients. The cognitive tests performed at 1 year showed better results in the TP arm; however the difference between 2 arms was not significant.

**Conclusion:** Patients with a history of abnormal-TCD had significantly lower cognitive performances than their siblings, even in absence of stroke history. There was no significant difference between the 2 arms for the outcomes of ischemic lesions, stenosis and cognitive performances. Despite the higher ability of HSCT to decrease velocities at 1 year compared to TP, a longer follow-up will be required to demonstrate its effect on stenosis and cognitive performances.  
**Disclosure of Interest:** None declared.

#### O080

##### **Hematopoietic Stem Cell Transplant for Thalassemia Intermedia: Single Center Experience**

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**Introduction:** Hematopoietic stem cell transplant (HSCT) to cure thalassemia major (TM) is an established modality but its role in management of thalassemia intermedia (TI) is still not very clear. Most of the centers use individualized approach for every patient which is quite apt looking at the phenotypic variability of TI. HSCT can be considered for patients who fall at the other spectrum of TI close to TM. We present our experience of HSCT in patients with TI.

**Material (or patients) and methods:** Twenty one patients diagnosed as TI (based on transfusion initiated after 2 years of age) underwent matched sibling HSCT. Mean age at diagnosis was 3.2 years, mean number of transfusions received before HSCT were 82 PRBC transfusions. Six patients were splenectomized whereas 15 had splenomegaly. All the patients were conditioned with iv busulfan 3.2mg/kg x 4days, iv cyclophosphamide 50mg/kg x 4 days and horse ATG (ATGAM) 30mg/kg x 3 days. GVHD prophylaxis included cyclosporine 3mg/kg in two divided doses starting D-3 and inj methotrexate 10mg/m<sup>2</sup> on D+1 followed by 7mg/m<sup>2</sup> on D+3,+6,+11. Bone marrow (BM) was used as a source in 15 whereas peripheral blood (PB) was used in 6 patients. Median CD34 cell dose was 3.68x10<sup>6</sup>cells/kg and median MNC count was 3.27x10<sup>8</sup>cell/kg.  
**Results:** Median polymorphonuclear cell and platelet engraftment were seen on D+ 14 and D+ 27.5 respectively. Whole blood chimerism on day+21 and day+60 were 83.40% and 73.97% donor cells. Median engraftment of polymorphonuclear cell and platelets in splenectomized patients was 11 days and 29 days respectively and 16 days and 27 days in the non splenectomized group. Chimerism in the splenectomized patients on day+21 and day+60 was 93.71% and 78.46% respectively whereas in the non splenectomized group was 78.98% and 72.04% respectively. Grade 2-4 GVH was seen in 4 patients (19.04%) whereas grade 3-4 was seen in 1 patient (4.8%). At a median follow up of 606 days the overall survival is 95.2% and disease free survival is 80.95% (1 expired, 2 rejected).

**Conclusion:** HSCT can safely be used as a curative modality for selected group of TI patients with good overall and event free survival. This option should be explored more often in TI so as to offer these patients better quality of life. From our observation splenectomized patients far well as compared to patients with splenomegaly undergoing HSCT.

**Disclosure of Interest:** None declared.

#### O086

##### **Graft storage and disposal policies in a large group of European centers: a survey by the cellular therapy and immunobiology working party (CTIWP) of the European Group For Blood And Marrow Transplantation (EBMT)**

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**Introduction:** There is considerable heterogeneity in processing of stem cells (SC) for transplantation (HSCT) across Europe. The FACT-JACIE International Standards have been developed to improve the quality of HSCT. At the same time they aim to avoid restrictions and leave some practices to the discretion of the individual transplant center (TC).

This survey aims to better understand the extent of heterogeneity in storage conditions, graft processing and disposal.

**Material (or patients) and methods:** A questionnaire regarding graft storage conditions, processing and disposal was developed and reviewed by the CTIWP of the EBMT. EBMT TC were invited to participate in this survey.

**Results:** 299 TC from 46 countries (32 European, 14 associated) responded to the survey. 231 (77%) questionnaires were complete. Fifteen TC (5%) do not currently have a SC processing facility.

73% of teams may store grafts overnight before freezing or infusion. Grafts are kept mostly at 4 ± 2°C. For long-term storage, 70% store at -175°C, either in liquid nitrogen (22%) or in vapour phase (38%). 26% of all responding TC store both in liquid and vapour nitrogen.

Potentially infectious grafts were mostly stored in an additional bag and in 54% in a separate tank. In case of microbiological contamination, most TC make a case-by-case decision in collaboration with the clinicians.

CD34 counts are performed according to ISHAGE standards either before or after thawing or both (54%) and are done using a single platform method in 81% of TC using commercially available kits (99%). This method is based on the use of a nucleic acid dye for identification of the necrotic cells in 74% of teams. Most of the teams (81%) report quality control results to the clinicians (98% CD34 counts, 59% CD3 counts and 29% report additional information).

Most TC (74%) preserve grafts using 10% DMSO and use a rate controlled freezing system (90%). The cells are not washed (83%), and are thawed at the bedside (68%) in a water bath (78%). There is a large heterogeneity between the TC regarding delivery to the transplant unit and the maximum delay within which thawed cells have to be infused.

79% of responding TC have a written policy for the handling of unused SC products, which is usually part of the patient information and consent document signed before collection. Time limitation on storage for autologous SC products exists in 42% of TC (in most TC limited to 5-10 years) and duration of storage is not disease or age dependent. For donor lymphocytes and directed cord blood products there is no limitation fixed for storage in 78% and 94% of TC, respectively. In cases where the product is disposed of and the patient is still alive, 70% of TC will inform the patient beforehand. Otherwise 88% of products are disposed of when a patient

dies with only 43% being used for research purposes. This information is made available to the patient via the information pack and consent forms in 91% of TC. However, in the case of allogeneic products consent for disposal is only requested in 46% of TC.

**Conclusion:** Overall, this survey demonstrated that the majority of responding TC use standard procedures for handling and processing of grafts. Some differences between TC exist, which requires discussion and more in depth analysis in order to improve graft and transplant outcome.

**Disclosure of Interest:** None declared.

#### O087

##### **Local injection of bone marrow progenitor cells for the treatment of anal sphincter injury: *in vitro* expanded versus minimally-manipulated cells**

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**Introduction:** Anal incontinence is a disabling condition that adversely affects the quality of life of a large number of patients, mainly with anal sphincter lesions. In a previous experimental work *in vitro* expanded bone marrow (BM) derived mesenchymal stem cells (MSC) were demonstrated to enhance sphincter healing after injury and primary repair in a rat preclinical model. Thus, in the present study, we investigated whether bone marrow mononuclear (MNC) unexpanded cells may also be effective.

**Material (or patients) and methods:** 32 Lewis rats, divided in four groups, underwent to sphincterotomy and surgical repair of anal sphincter plus intrasphincteric injection of 1) saline (CTR), 2) *in vitro* expanded MSC (MSC), 3) minimally manipulated MNC (MNC); moreover, the fourth group underwent sham operation. At day 30 histologic and morphometric analysis and *in vitro* contractility functionality were performed.

**Results:** Treatment with both *in vitro* expanded MSC and minimally manipulated MNC improved muscle regeneration and increased contractile function of anal sphincters after injury and primary repair compared to CTR. Contractile ability of sphincter smooth muscle of MNC-treated and MSC-treated animals were comparable upon electrical as well as chemical stimulation and both were significantly higher ( $P < 0.05$ ) at day 30 after sphincterotomy than the untreated animals. Histologic analysis showed that in rats treated with BM derived stem cells (MSC or MNC) the injured area appeared almost completely repaired one month after sphincterotomy. Results of morphometric analysis showed that muscle area fraction (MAF) was significantly increased in rats treated with BM MSC or MNC as compared to controls ( $67.15 \pm 9.8$ ;  $70.63 \pm 11.7$  % respectively, vs  $26.438 \pm 6.6\%$ ,  $P < 0.01$ ). No significant difference were observed between the two BM stem cell source used. GFP+ cells (MSC and MNC) remained in the proximity of the lesion site up to 30 days post injection. No co-localization of GFP and smooth muscle cells marker  $\alpha$ SMA has ever been observed.

**Conclusion:** In the present study we demonstrated in a preclinical model that minimally manipulated BM-MNC were as effective as *in vitro* expanded MSC for the recovery of anal sphincter injury followed by primary sphincter repair. These results may have great impact for improving clinical applications of stem cell therapy in human anal incontinence treatment.

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**Disclosure of Interest:** None declared.

#### O088

##### **Granulocyte colony-stimulating factor mobilization increases the proportion and immunosuppressive function of regulatory $\gamma\delta$ T cells**

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**Introduction:** The immune modulatory effect of granulocyte colony-stimulating factor (G-CSF) on T cells resulted in an unexpected low incidence of graft-versus-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation. Our previous studies demonstrated that G-CSF mobilization influenced the distribution and clonality of TRGV and TRDV repertoire (T cell receptors of  $\gamma\delta$  T cells)<sup>1</sup>. Regulatory  $\gamma\delta$  T cells ( $\gamma\delta$  Tregs), which express Foxp3 and primarily belong to CD27<sup>+</sup> CD25<sup>high</sup> phenotype, are a novel subset of cells with immunosuppressive function<sup>2</sup>. However, whether G-CSF mobilization could influence the expression and immunosuppressive function of  $\gamma\delta$  Tregs remains unknown.

**Material (or patients) and methods:** Peripheral blood  $\gamma\delta^+$  T cells were sorted by magnetic activated cell-sorting (MACS) from 10 donors before and after G-CSF mobilization, respectively. MACS-sorted  $\gamma\delta$  T cells were used as effector cells in the carboxylfluorescein diacetate succinimidyl ester (CFSE) assays and cell immunophenotyping was analyzed by flow cytometry. Autologous CD4<sup>+</sup> T cells were purified by MACS, labeled with CFSE, used as responder cells, and finally co-cultured with effector cells. After 5 d incubation, cells were harvested and analyzed for flow cytometry by gating on the CFSE-labeled cells. The percentages of divided responder T cells before and after G-CSF mobilization were compared.

**Results:** Compared with that before mobilization, the proportions of Foxp3<sup>+</sup> $\gamma\delta$  T, Foxp3<sup>+</sup> V $\delta$ 1 T and Foxp3<sup>+</sup> V $\delta$ 2 T cells were significantly increased in sorted  $\gamma\delta$  T cells after G-CSF mobilization ( $P = 0.035$ ,  $P = 0.017$  and  $P = 0.004$ ). The proportions of CD27<sup>+</sup> $\gamma\delta$  T, CD27<sup>+</sup>V $\delta$ 1 T and CD27<sup>+</sup>V $\delta$ 2 T cells were also significantly increased in sorted  $\gamma\delta$  T cells after G-CSF mobilization ( $P = 0.035$ ,  $P = 0.026$ ,  $P = 0.037$ ). The proportions of CD25<sup>+</sup> $\gamma\delta$  T, CD25<sup>+</sup> V $\delta$ 1 T and CD25<sup>+</sup>V $\delta$ 2 T cells were similar in sorted  $\gamma\delta$  T cells before and after mobilization ( $P = 0.252$ ,  $P = 0.126$ ,  $P = 0.237$ ). We then compared the inhibitory effect of sorted  $\gamma\delta$  T cells before and after G-CSF mobilization on the proliferation of autologous CD4<sup>+</sup> T cells. We found that MACS-sorted  $\gamma\delta$  T cells before and after G-CSF mobilization both exerted certain suppression on proliferation, whereas  $\gamma\delta$  T cells after G-CSF mobilization had a more significant suppressive effect on CD4<sup>+</sup> T cells than  $\gamma\delta$  T cells before mobilization ( $P = 0.002$ ).

**Conclusion:** G-CSF mobilization could increase the proportions of regulatory T cells in sorted  $\gamma\delta$  T cells, thus having a higher suppressive effect on CD4<sup>+</sup> T cells.

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**Disclosure of Interest:** None declared.

O089

**Removal of  $\alpha\beta$  T-cells and B-cells for HLA-haploidentical HSCT: procedure robustness and reliability after three-year experience**

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**Introduction:** Haploidentical hematopoietic stem cells transplantation (HSCT) is an effective procedure for treatment of both malignant and non-malignant hematological diseases when a suitable HLA-matched donor is lacking. In order to reduce the incidence of GvHD, we used an immunomagnetic depletion method, which selectively removes  $\alpha\beta$  T-cells. B-cells were also depleted to prevent post-transplant EBV-associated lymphoproliferative disease. The depleted cell fraction infused to the patients contains, in addition to CD34 HSC,  $\gamma\delta$  T cells and NK cells that may contribute to immune reconstitution and to the GvL effect. Using this approach, we have previously reported a robust engraftment and rapid immune reconstitution in children with non-malignant diseases (Bertaina et al., Blood 2014). Here, we describe our three-year experience with 170 procedures assessing depletion efficiency, cell recovery and reliability over time.

**Material (or patients) and methods:** Haploidentical related donors received G-CSF from day -5 to -1 for mobilization prior to leukapheresis (LA). LA (170 procedures from 165 donors) were performed when CD34-cell counts were  $\geq 40/\mu\text{l}$ . Plerixafor was given to 43 donors (26%) 9 hrs before LA when CD34 count was  $< 40/\mu\text{l}$  the day before LA. LA products containing up to  $60 \times 10^9$  WBC were processed in a closed bag system with a CliniMACS device (Miltenyi, Bergish Gladback, DE) for depletion of  $\alpha\beta$  T-cells using a biotinylated anti-TcR  $\alpha\beta$  Ab followed by an anti-biotin Ab conjugated to paramagnetic beads. Concomitantly, B-cells were also depleted by using anti-CD19 Ab conjugated to paramagnetic beads. The cell products (grafts) were characterized for both residual  $\alpha\beta$  T-cells and B-cells to determine depletion efficiency. The grafts were reinfused within 4hrs after completion of processing in children with both hematological malignant and non-malignant diseases.

**Results:** The median recovery of CD34 HSC was 98.2% (range 67-115) and the median number of infused CD34 HSC was  $18.3 \times 10^6/\text{kg}$  (range  $6.7\text{-}40.5 \times 10^6/\text{kg}$ ). The mean depletion of  $\alpha\beta$  T-cells was  $4.18 \log \pm 0.48$  with a median number of transplanted  $\alpha\beta$  T-cells of  $0.030 \times 10^6/\text{kg}$  (range 0.001-0.11). The mean depletion of B-cells was  $3.5 \log \pm 0.49$  with a median number of transplanted B-cells of  $0.035 \times 10^6/\text{kg}$  (range 0.002-0.92). Median yield of NK and  $\gamma\delta$  T cells was 90.7% (range 33-111) and 88.3% (range 34-112), respectively. The median number of NK cells infused was  $31.4 \times 10^6/\text{kg}$  (range 3.8-179) while that of CD3 T-cells was  $10.3 \times 10^6/\text{kg}$  (range 1.3-106), made up mostly by  $\gamma\delta$  T-cells (median number infused  $10.1 \times 10^6/\text{kg}$ , range 0.9-104). In order to assess the robustness and reliability of the procedure, the same parameters were evaluated at 6-month intervals over a 3-year period. Steady performance of the procedure was observed, with minimal fluctuations that did not reach statistical significance and showed no drift during the three-year follow-up.

**Conclusion:** The procedure for removal of  $\alpha\beta$  T-cells and B-cells is reliable with remarkable degree of depletion attained in all cases. The grafts contained high numbers of CD34 HSC and of immune effector  $\gamma\delta$  T cells and NK cells. Depletion efficiency and cell recovery monitored over time were stable, suggesting that the personnel is properly trained and that different batches of reagents and disposables guarantee consistent performances.

**Disclosure of Interest:** None declared.

O090

**Transplantation of Ex vivo Expanded Umbilical Cord Blood (NiCord) Results in Decreased Infection Burden and Hospital Length of Stay in the First 100 Days**

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**Introduction:** Delayed hematopoietic and immunologic recovery contributes to increased risk of infection following umbilical cord blood (UCB) transplantation. Adult recipients of UCB stem cells that were expanded ex vivo for 3 weeks in the presence of nicotinamide (NiCord) had an earlier median neutrophil recovery compared to historical controls<sup>1</sup>. Therefore, we evaluated whether NiCord provides clinical benefit in the form of reduced risk of infection and decreased hospital length of stay (LOS) through day +100.

**Material (or patients) and methods:** We retrospectively reviewed infection episodes and hospital LOS in 18 adult recipients of NiCord grafts; 10 patients received NiCord with a second unmanipulated unit and 8 patients received NiCord as a single UCB graft. Outcomes were compared to 101 consecutive adult recipients of standard single or double UCB transplantation at Duke University from January 2005 to March 2015. All patients had hematologic malignancy (acute leukemia/MDS 90%, lymphoid 10%) and received a total body irradiation-based myeloablative preparative regimen. Patients were considered at risk of early infection until day +100, relapse, second transplant, death, or last follow-up, whichever came first. Infectious episodes were categorized by type (bacterial, viral, fungal, non-microbiologically defined) and severity (grade 1, 2, 3)<sup>2</sup>. Infection density over time was assessed with Poisson regression. Hospital LOS, defined as "days alive and out of the hospital in the first 100 days" to account for early deaths<sup>3</sup>, was assessed with ANCOVA.

**Results:** Overall, median time to neutrophil engraftment in NiCord patients was 12.5 days (95% CI 10-18), significantly shorter than 27 days (95% CI 23-28) in standard UCB patients (log-rank  $P < .001$ ). All 18 NiCord and 100 of 101 standard UCB patients had at least one infection of any severity. Nine of 18 NiCord and 62 of 101 standard UCB patients had at least one grade 2-3 (moderate to severe) infection ( $P = .364$ ). Pathogen-specific comparison of grade 2-3 infection in NiCord versus standard UCB recipients showed significantly lower bacterial infection frequency 22% vs. 54% ( $P = .015$ ), respectively, but no difference in viral (39% vs. 35%,  $P = .729$ ), fungal (0% vs. 5%,  $P = 1.0$ ), or non-microbiologically defined (0% vs. 17%,  $P = .072$ ) infections. Univariate analysis revealed significantly reduced risk ratio for all bacterial infection (0.35,  $P = .001$ ) and grade 2-3 non-viral infection (0.16,  $P < .001$ ) in NiCord versus standard UCB patients; this effect was largely unchanged after multivariate adjustment for age, disease stage (early or non-early), and acute grade 3-4 GVHD. NiCord patients had 21.51 (95% CI 8.25, 34.78) more days alive and out of the hospital in the first 100 days than standard UCB patients ( $P = .002$ ) after adjustment for age, KPS, and acute grade 3-4 GVHD.

**Conclusion:** Transplantation of NiCord was associated with reduced incidence of all bacterial infection and moderate to severe non-viral infection compared to standard UCB transplantation in the first 100 days. NiCord recipients also had significantly decreased hospital length of stay in the first 100 days. Our results confirm that rapid hematopoietic recovery from an ex vivo expanded UCB transplantation approach results in clinical benefit.

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2. BMTCTN Technical MOP version 3.0, Appendix 4-A.

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#### O091

##### Single Cell Tbet/Foxp3 Regulation of Autoimmune GVHD

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**Introduction:** Allogeneic Bone Marrow Transplantation (BMT) is the primary therapy for poor prognosis leukemia's, relapsed lymphomas and primary immune deficiency syndromes but >50% of patients develop autoimmune chronic graft versus host disease (cGVHD) <sup>1</sup>. Unlike acute GVHD, cGVHD remains a major cause of morbidity and mortality<sup>2-4</sup> post BMT. CD4<sup>+</sup> T cell subsets are primary mediators of both acute and chronic GVHD with T-bet<sup>+</sup> Th1, GATA3<sup>+</sup> Th2 and RORγt<sup>+</sup>Th17 cell subsets mediating acute and chronic GVHD<sup>5</sup>. In contrast, CD4<sup>+</sup> FoxP3<sup>+</sup> regulatory T cell subsets play a prominent role in preventing GVHD<sup>6</sup>. However, it remains unclear how specific T-helper subsets contribute to cGVHD and the molecular mechanisms that are involved in inhibiting Treg generation in cGVHD.

**Material (or patients) and methods:** Using a murine model to focus on autoimmune components of GVHD, we evaluated if T helper Type 1 signaling deficient donor Th1 cells inhibit FoxP3-driven regulatory T cell (Treg) peripheral generation in cGVHD. Briefly, irradiated host mice (Balb/c) were reconstituted with fully mismatched T cell depleted bone marrow from C57BL6 mice along with CD4<sup>+</sup> T helper cells. This constituted the first phase of aGVHD. At day 21, the alloreactive WT or *IFNγR*<sup>-/-</sup> or *Tbet*<sup>-/-</sup> T cells were isolated and then transferred into *B6.Rag2*<sup>-/-</sup> recipients<sup>7</sup>.

**Results:** Murine recipients that received WT T cells developed severe skin and colitic manifestations of cGVHD while the recipients of *IFNγR*<sup>-/-</sup> T cells survived, showed no clinical manifestations of cGVHD and had increased Treg cell numbers at day 60 post-transplant. Similarly, adoptive transfer of Tbet deficient T cells resulted in murine recipients developing aGVHD and had similar Treg numbers as compared to WT cohorts (0.40% vs. 0.17%, respectively; *P*=NS). During cGVHD, murine recipients reconstituted with *Tbx21*<sup>-/-</sup> (*Tbet*<sup>-/-</sup>) T cells had a significant survival benefit compared to their WT counterparts and had significantly elevated Treg cell numbers. To evaluate whether peripheral induction contributed to regulatory T cell predominance, we adoptively transferred *Tbx21*<sup>-/-</sup> T cells that consisted of fate mapping for FoxP3: recipients of flow-purified effector cells that were Foxp3<sup>+</sup> and *Tbx21*<sup>-/-</sup> had enhanced Treg predominance during autoimmune GVHD, thus directly demonstrating that peripheral Treg induction is controlled by Tbet. *Tbx21*<sup>-/-</sup> T cells also cross-regulated autoimmune GVHD mediated by otherwise pathogenic wild-type T cells.

**Conclusion:** The Tbet pathway therefore directly impairs Treg reconstitution and is therefore a feasible target in efforts to prevent autoimmune GVHD.

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#### O092

##### Treg-induced NOTCH1 blockade on conventional T cells act through the cd39/adenosine axis to control GVHD

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**Introduction:** Despite growing interest in T regulatory cells (Tregs), their mechanisms of action are still under debate. NOTCH signalling plays an essential role in regulating alloimmune T cell responses that mediate graft-versus-host-disease (GvHD), so in the present study we investigated if Treg can modify NOTCH signalling in conventional T cells (Tcons), thus impacting on GvHD and lethality.

**Material (or patients) and methods:** Tregs were co-cultured with CFSE<sup>+</sup>Tcons w/o antiCD39 mAb. After 5 days, Tcons were analysed for NOTCH1 expression. Sorted CFSE+Tcons were used for suppression assays and mRNA quantification. BALB/c mice received 5x10<sup>5</sup> donor type (C57BL/6) GFP<sup>+</sup> Treg on day -2, 5x10<sup>6</sup> T cell depleted bone marrow cells and 1x10<sup>6</sup> Tcon from C57BL/6 mice on day 0. In each experiment a group of mice were treated with the selective CD39 inhibitor polyoxometalate-1 (POM1).

**Results:** NOTCH1 and HES1 mRNA expression were reduced in Tcons when co-cultured with Tregs (to 66.5 ± 20%, and 71 ± 34%, respectively vs Tcons alone). Utilizing flow cytometry, a significant decrease in the NOTCH1 receptor expression (7.7% ± 3.8 vs 2.8 ± 1.6 ; p\*\*\* = 0.0009) and in NOTCH1-intracellular domain (NICD) (4% ± 2.37 vs 1.73 ± 1.6 ; p\* = 0.015) was observed. Adding the anti-CD39-blocking mAb to Treg/Tcon co-cultures rescued NOTCH1 receptor (2.8% ± 1.6 vs 5.5% ± 2.5; p\*\* = 0.0095) and NICD expression (1.73% ± 1.6 vs 6.2% ± 4.5; p\*\* = 0.0047). Further the addition of CD39 MoAbs resulted in a significantly reduced Treg-suppressive capacity from 56% ± 4.5 to 40.3% ± 2 (p\*\* = 0.0058). When mice received Tregs + Tcons NOTCH1 expression on CD4<sup>+</sup>- and CD8<sup>+</sup>-H2K<sup>b</sup>GFP<sup>+</sup> cells from lymph nodes was significantly reduced when compared with Tcons alone (8.8 ± 1.6% vs 3 ± 1.5% \**P* < 0.05 and 19 ± 2.8% vs 6.6 ± 2.8% \**P* < 0.05). The addition of POM1, which inhibits CD39, resulted in restoration of NOTCH1 expression to 13.7 ± 0.9% \*\**P* < 0.001 on CD4<sup>+</sup>-H2K<sup>b</sup>GFP<sup>+</sup> cells and 21.9 ± 2.6% on CD8<sup>+</sup>-H2K<sup>b</sup>GFP<sup>+</sup> cells \**P* < 0.05, approaching the same level observed on the mice that received Tcons alone. As expected mice that received Tregs + Tcons had improved survival in comparison with mice that received Tcons alone (\**P* < 0.05). Animals treated with Tregs +Tcons had reduced incidence of GvHD (\*\**P* < 0.01) and mice had improved weight gain (\*\**P* < 0.01). In mice that received Tregs and Tcons and POM1, survival was reduced (\**P* < 0.05), with an increase in the incidence of GvHD (\*\**P* < 0.01) and the mice did not regain weight (\*\**P* < 0.01 vs Group 4)

**Conclusion:** The present paper describes for the first time a Treg-induced NOTCH1 blockade. Tregs trigger and orchestrate NOTCH downregulation directly in Tcons. They act through the CD39/adenosine axis to inhibit the NOTCH pathway which, in turn, regulates Tcon proliferation. NOTCH1 inhibition on Tcons could explain clinical and experimental evidences from our group showing that Tregs prevent GvHD and allow for a powerful Tcon-dependent GvL. Consequently Treg-mediated NOTCH inhibition may represent a new mechanism to separate GvHD from GvL. This finding has major implications for adoptive immunotherapy strategies in the field of transplantation for leukaemia.

**Disclosure of Interest:** None declared.

O093

### The role of Cathepsin E in GVHD

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**Introduction:** The interaction of microbial products and their sensing receptors is known to be involved in antigen presentation. In preclinical models, we have demonstrated that signaling through the pathogen recognition receptor NOD2 regulates GVHD by its influence on antigen presentation and allo-activation of donor T-cells.

**Material (or patients) and methods:** We used NOD2<sup>-/-</sup> C57B6 mice, Ctse<sup>-/-</sup> C57B6 and wild type (WT) C57B6 as donors or recipients in preclinical GVHD models (LP/J in C57B6; C57B6 in B6D2F1, C57B6 in Balb/c).

**Results:** To identify pathways that are related to NOD2 signaling during GVHD we performed gene array analyses in NOD2<sup>-/-</sup> allo-BMT recipients vs. WT allo-BMT recipients. Most striking was a 13fold expression difference of Cathepsin E (ctse) between NOD2<sup>-/-</sup> allo BMT recipients vs. WT allo-BMT recipients during hepatic GVHD. Cathepsin E (ctse) is an aspartate protease expressed in dendritic cells and macrophages which regulates cleaving of bacterial peptides for antigen presentation by the MHC II complex. Recent data in Ctse<sup>-/-</sup> mice demonstrated reduced inflammation in allergic disease models. Using ctse<sup>-/-</sup> T-cells and ctse<sup>-/-</sup> donor bone marrow (BM) cells vs WT donor cells, we detected no major impact of ctse donor deficiency on GVHD and engraftment. In contrast, ctse<sup>-/-</sup> allo-BMT recipients had significantly reduced GVHD-related mortality and significantly lower clinical GVHD scores as compared to WT allo-BMT recipients (Fig.1). Our results demonstrate that T-cell proliferation *in vivo* and *in vitro* is significantly decreased in ctse-deficient allo-BMT recipients during GVHD. Accordingly, ctse-deficient APCs had a lower ability to induce allo-activation of WT T-cells.

**Conclusion:** We conclude that ctse is a relevant factor during GVHD. Therapeutic targeting of aspartate proteases is feasible and may be a future option to inhibit inflammation.

**Disclosure of Interest:** None declared.

O094

### The Chemerin/Chemr23 axis plays a pivotal role in the pathogenesis of intestinal damage in a murine model of Graft-versus-Host Disease

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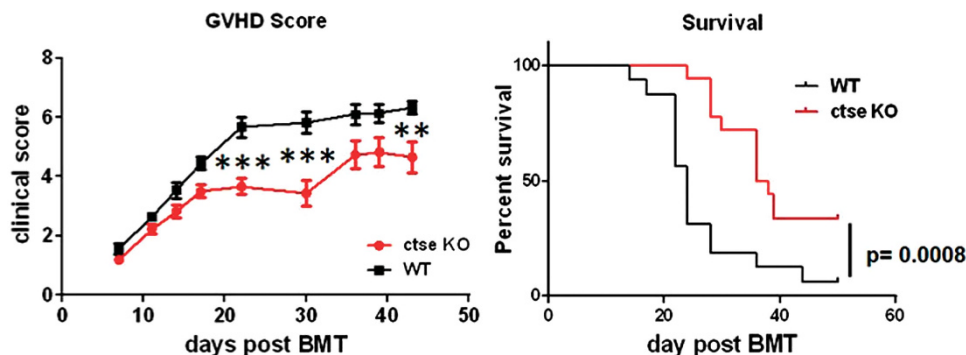
**Introduction:** Graft-versus-Host Disease (GvHD), represents the major cause of mortality and morbidity after Hematopoietic Stem Cells Transplantation. Chemerin has been recently identified as a chemotactic protein, which is produced by several tissues during inflammation and binds the G protein-coupled receptor ChemR23, expressed by DCs, macrophages and NK cells. The aim of this study was to evaluate the potential role of Chemerin/ChemR23 axis in the pathogenesis of GvHD, in order to identify disease-specific pathways exploitable for developing new potential therapeutic targets.

**Material (or patients) and methods:** Lethally irradiated Balb/C recipient mice were transplanted with bone marrow cells and splenocytes obtained from ChemR23-deficient C57BL6 mice (tChemR23<sup>-/-</sup>). After transplantation, mice were monitored daily for survival and GvHD severity. Recipient mice were sacrificed at different time points to evaluate Chemerin production and leukocytes infiltration in GvHD target organs by using different techniques, such as ELISA, histopathology, FACS and PCR.

**Results:** Starting from day +6 after transplantation, Chemerin plasma levels appeared significantly higher in both wild type (WT) and tChemR23<sup>-/-</sup> mice who developed GvHD, compared to syngeneic controls. Interestingly, tChemR23<sup>-/-</sup> mice developed a more severe GvHD compared to mice transplanted with WT cells. In particular, tChemR23<sup>-/-</sup> mice showed a higher mortality rate. Differences in GvHD score between ChemR23<sup>-/-</sup> and WT transplanted mice resulted by a significantly increase in weight loss, associated to severe diarrhea. In accordance, histological analysis performed on GvHD target organs showed a significantly higher GvHD score in large intestine of tChemR23<sup>-/-</sup> mice, whereas no differences were found in other GvHD organs. In addition, a deeper histological analysis on large intestine showed that tissue damage is characterized by crypt hyperplasia and atrophy, epithelium apoptosis and colitis.

FACS analysis of large intestine infiltrating leukocytes showed that the percentage of neutrophils infiltrating colon were significantly higher in tChemR23<sup>-/-</sup> mice compared to WT transplanted mice. The higher neutrophils infiltration was also confirmed by immunohistochemistry evaluating the number of myeloperoxidase (MPO) positive cells and MPO expression by RQ-PCR. Interestingly, the analysis of ChemR23<sup>+</sup> cell subsets revealed that macrophages infiltrating colon mucosa were significantly lower in tChemR23<sup>-/-</sup> mice compared to WT, while no differences were observed in DCs or NK cells. All these

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**Figure 1:** Clinical GVHD score and approximate survival of ctse-deficient allo-BMT recipients compared to wildtype littermates. Ctse KO n=18, WT n=15. **A)** Cumulated clinical score of ctse KO and WT littermates post BMT. \*\* <0.01, \*\*\* <0.001. **B)** Approximate survival curve of ctse KO and WT allo-BMT recipients. Combined data from two experiments are shown.

observations were also obtained by analyzing the mesenteric lymphnode. By adoptively transferring  $0.2 \times 10^6$  WT-monocytes into tChemR23<sup>-/-</sup> mice, we demonstrated that WT-monocytes were able to improve GvHD in terms of survival, weight loss and overall score, thus confirming that lacking of ChemR23 expression on macrophages induces an increase of GvHD damage.

**Conclusion:** All these findings suggest that the Chemerin/ChemR23 axis plays a crucial role in intestinal GVHD, driving macrophages infiltration in colon mucosa. Further studies are needed to better understand the mechanisms underlying the severe damage observed in the gastrointestinal tract of tChemR23<sup>-/-</sup> mice.

**Disclosure of Interest:** None declared.

**O095**

**Dissecting the mechanisms involved in anti-human T-lymphocyte immunoglobulin (ATG)-induced tolerance in the setting of allogeneic stem cell transplantation - potential implications for GVHD**

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**Introduction:** Polyclonal anti-human T-lymphocyte immunoglobulin (ATG) have been recently shown, in two randomized studies, to significantly reduce the incidence of graft versus host disease (GVHD) post allogeneic stem cell transplantation (HSCT) from both sibling and unrelated donors. Induction of

regulatory T cells is suggested as one of the possible mechanisms involved.

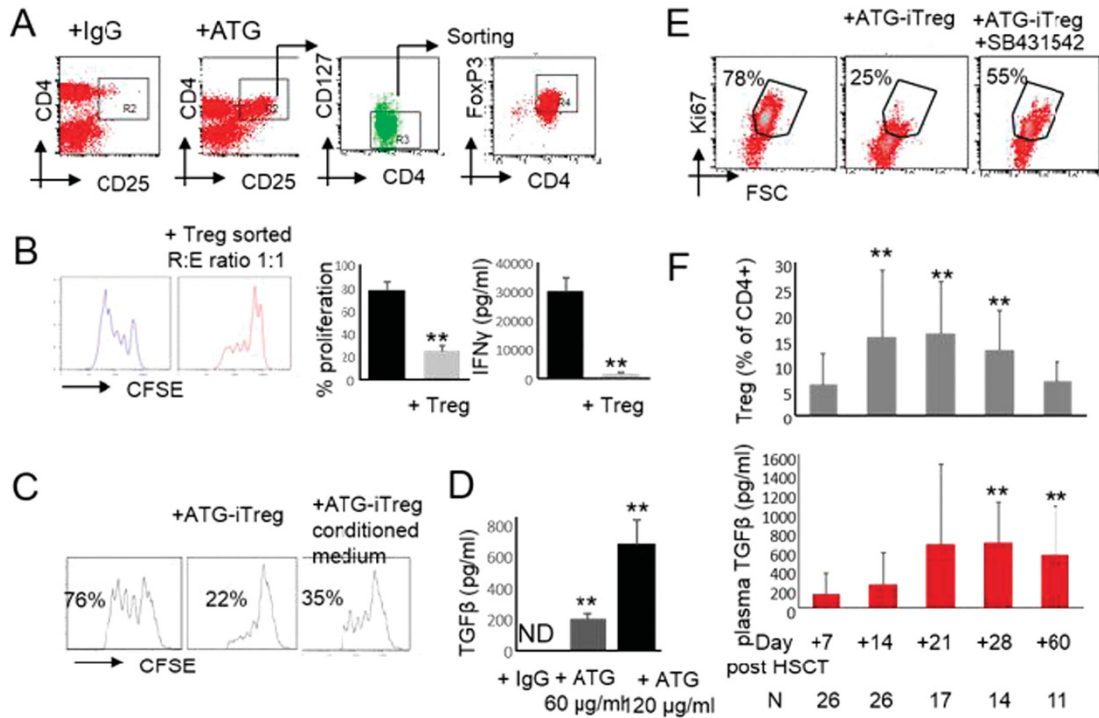
**Material (or patients) and methods:** The aim of our current study was to further characterize the T cell populations induced by ATG treatment and to delineate the mechanisms involved in ATG-induced tolerance in patients receiving intravenous ATG (Neovii Biotech) as part of their pre HSCT conditioning.

**Results:** Ex vivo treatment with ATG (30-120 µg/ml) resulted in significant increase in regulatory markers CD25, CTLA-4, GITR, CD95, ICOS, PD-1 and FoxP3 expression on CD4+ T cells. In addition, expression of CD127 and VLA-4 was significantly decreased on CD4+CD25+ cells upon ATG treatment ( $P < 0.01$ ). Addition of ATG-treated cells to autologous PBMCs stimulated with antiCD3/antiCD28 antibodies resulted in significant inhibition of cell proliferation, CD69 expression and interferon-γ (IFNγ) secretion ( $P < 0.001$ ). Importantly, addition of cyclosporine A to the induction culture with ATG interfered with the ATG-induced regulatory phenotype acquisition, suggesting the involvement of interleukin-2 in ATG-mediated activity.

In order to purify the tolerizing population, sorting of CD4+CD25+CD127-low cells (considered as viable Treg cells) from ATG-treated culture was performed. Sorted cells demonstrated greater suppressive potency than bulked pre-sorted cell population.

To explore the possible involvement of soluble factor(s)-mediated mechanisms, conditioned medium (CM) produced by ATG-primed cells was applied on stimulated autologous PBMCs. It resulted in significant suppression ( $P < 0.01$ ) of T cell proliferation and activation, indicating the presence of soluble factors secreted by ATG-primed suppressive cells. Indeed, significant dose- and time-dependent induction of TGFβ secretion was observed in ATG-treated cells. Addition of TGFβ receptor kinase inhibitor SB-431542 interfered with

[O095]



(A) Sorting of ATG-induced CD4+CD25+CD127-low cells. Foxp3 is expressed by 95% of sorted cells. (B) Proliferation and activation of PBMCs stimulated with anti-CD3/anti-CD28, in the absence or presence of autologous ATG-induced sorted Treg cells. (C) Proliferation of stimulated PBMCs in the absence or presence of ATG-induced Treg cells or conditioned medium produced by Treg cells. (D) TGFβ secretion upon ATG in vitro treatment. (E) Effect of TGFβR kinase inhibitor SB431542 on proliferation of stimulated PBMCs in the absence or presence of ATG-induced Treg cells. (F) Frequency of circulating Treg cells and plasma levels of TGFβ in patients undergoing allogeneic HSCT with ATG-including conditioning (\*\*p<0.01).

suppressive activity of ATG-primed cells, enabling partial rescue of proliferation and IFN $\gamma$  secretion in response to TCR activation.

Finally, characterization of phenotype and frequencies of regulatory immune populations in peripheral blood of 26 patients undergoing allogeneic transplantation with conditioning regimen including ATG (15mg/kg) was performed. Consistent with our ex vivo results, transient increase in percent of circulating CD4+CD25+CD127-low cells was detected in the ATG treated patients on day 21 after HSCT. Furthermore, elevated levels of TGF $\beta$  were detected in the patients' plasma at day 28 and remaining high at day 60 post HSCT.

**Conclusion:** Our results demonstrate that *in vitro* treatment with ATG is capable to induce functional Treg cells. Suppressive ability of ATG-induced cells was partially promoted by TGF $\beta$  signaling. Patients undergoing allogeneic HSCT with ATG-including conditioning demonstrated increased frequencies of circulating Treg cells and elevated plasma levels of TGF $\beta$ . Altogether, our data further support the use of ATG, a potent inducer of regulatory T cells, for prevention of GVHD post HSCT and potentially other therapeutic applications.

**Disclosure of Interest:** K. Beider: None declared, D. Naor: None declared, V. Voevoda: None declared, O. Ostrovsky: None declared, H. Bitner: None declared, E. Rosenberg: None declared, N. Bloom: None declared, T. Simone: None declared, I. Danilesko: None declared, A. Shimoni: None declared, A. Nagler Funding from: Academic research grant from Neovii Biotech (IIT-ATG-28 Nagler Treg).

#### O096

##### **An open-label, single-group, phase 2 study of Brentuximab Vedotin as salvage therapy for males with relapsed germ-cell tumors (GCT): results at the end of first stage (FM12GCT01)**

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**Introduction:** Prognosis of patients (pts) failing multiple chemotherapy (CT) regimens is quite dismal. CD30 is expressed and prognostic in embryonal carcinoma, hence it is a rational target for treatment. Brentuximab Vedotin (BV) is an antibody-drug conjugate consisting of the chimeric anti-CD30 antibody SGN-30 conjugated to an antitubulin synthetic analog (MMAE). A phase 2 trial is ongoing in GCT (NCT01851200).

**Material (or patients) and methods:** 24 pts with biopsy-proven CD30+ GCT will receive BV 1.8 mg/Kg IV q3 weeks until disease progression or onset of unacceptable toxicity. Eligibility will include failure of 2 or 3 platinum-based CT (including HDCT). All pts will undergo measurement of serum tumor markers (STM), a computed tomography and a PET scan q6 weeks. An optimal Simon's 2-stage design will be applied. The primary endpoint is the objective response-rate (ORR; H0:  $\leq$  5%, H1:  $\geq$  25%,  $\alpha$  and  $\beta$  = 10%). In stage 1, 9 evaluable patients will be accrued. Sequential peripheral blood samples are being collected for immune profiling by flow cytometry of immune cell subsets.

**Results:** From 07/13 to 04/15, 9 pts have been treated, 3 in third-line, and 6 beyond the third-line. 5 had received HDCT. STM decline was obtained in 7 pts (77.8%) after the first dose and in 4 pts (44.4%) after 2 doses (with normalized [1] or still positive [3] STM). ORR was 22.2% (1 CR+1 PR) according to RECIST v1.1, and there were 3 metabolic PR.

3-month PFS was 11.1% (95%CI: 0.6-38.8), 6-month OS was 85.7% (95%CI: 33.4-97.9). 1 case of reversible G3

hyperglycemia was recorded. No BV discontinuation for toxicity occurred.

In 7 evaluable pts, a trend to increasing expression of activation (HLA-DR), proliferation (Ki67), and functional differentiation (Granzyme B) markers in CD4 and CD8 T cells was observed during BV. In CR patient, an increased frequency of naive and central memory T cells was observed after the first cycle of BV, as well as a reduction in the fraction of PD1<sup>+</sup>/CD8<sup>+</sup> T cells.

**Conclusion:** Brentuximab is endowed with potent antitumor activity and meaningful immunomodulatory effects in GCT. The rapid development of resistance is a concern. ORR is pending confirmation in the whole sample size.

**Disclosure of Interest:** None declared.

#### O097

##### **The survey on cellular and engineered tissue therapies in Europe in 2013**

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**Introduction:** Following the coordinated efforts of five established scientific organizations, this survey describes activity in Europe for the year 2013 in the area of cellular and engineered tissue therapies, excluding hematopoietic stem cell treatments for the reconstitution of hematopoiesis.

**Material (or patients) and methods:** Participating teams were requested to report their data for 2013 by indication, donor type, cell source, processing method and delivery mode using a survey which followed the traditional principles of the EBMT transplant activity survey and concentrates on numbers of patients with a first cellular therapy. 687 teams known to be actively transplanting in 48 countries were contacted for the 2013 EBMT survey, to which were added members of the other participating societies and teams who had contributed to any earlier survey.

**Results:** 318 teams from 31 countries responded to the cellular and engineered tissue therapy survey; 145 teams from 25 countries reported treating 2187 patients, while a further 173 teams reported no activity. Data on 6 patients were excluded from the analysis. Of the remaining 2181 patients, 629 (30%) were treated with allogeneic and 1552 (71%) with autologous cells. Indications were musculoskeletal/rheumatological disorders (45%; 89% autologous), cardiovascular disorders (20%; 99% autologous), hematology/oncology, predominantly prevention or treatment of GVHD and HSC graft enhancement, (19%; < 1% autologous), neurological disorders (3%; 100% autologous), gastrointestinal disorders (2%; 32% autologous) and other indications (11%; 67% autologous). The majority of autologous cells (88%) were used to treat musculoskeletal/rheumatological (57%) and cardiovascular (27%) disorders, whereas allogeneic cells were used mainly for hematology/oncology (64%). Cartilage and bone repair were by far the most frequently reported indications amongst the *musculoskeletal/rheumatological disorders*, comprising almost half of all treatments in this group, followed by reconstructive surgery/tissue enhancement (21% of treatments). Treatments for decubitus and leg ulcers were the main reasons for a cellular or engineered tissue therapy amongst the *cardiovascular disorders*, closely followed by peripheral artery disease, together accounting for 62% of treatments in this group of indications. The number of patients treated for *neurological and gastrointestinal indications* was small; Crohn's disease, multiple sclerosis and amyotrophic lateral sclerosis. The cell types were mesenchymal stem/



stromal cells (49%), hematopoietic stem cells (28%), chondrocytes (11%), dendritic cells (2%), keratinocytes (1%) and others (9%).

**Conclusion:** The data collected in this survey show a modest increase in both the number of reporting teams and number of patients treated from the previous year (143 in 2008 and 318 in 2013), with data reported from 33 in 2008 to 142 in 2013. The number of patients treated has risen from 1040 in 2008 to 2187 in 2013. When comparing the results for specific indications with previous years we found few significant differences. The international nature of the involved societies suggests for widening the data collection to other world regions, with global repository of data. A larger collective effort will be necessary in order to show that cell-based and tissue engineered therapies are, despite the challenges to be overcome, global opportunities to counteract still lethal diseases and unmet clinical needs.

**Disclosure of Interest:** None declared.

**O098**

**CART-NKG2D cells target osteosarcoma *in vitro* and *in vivo***

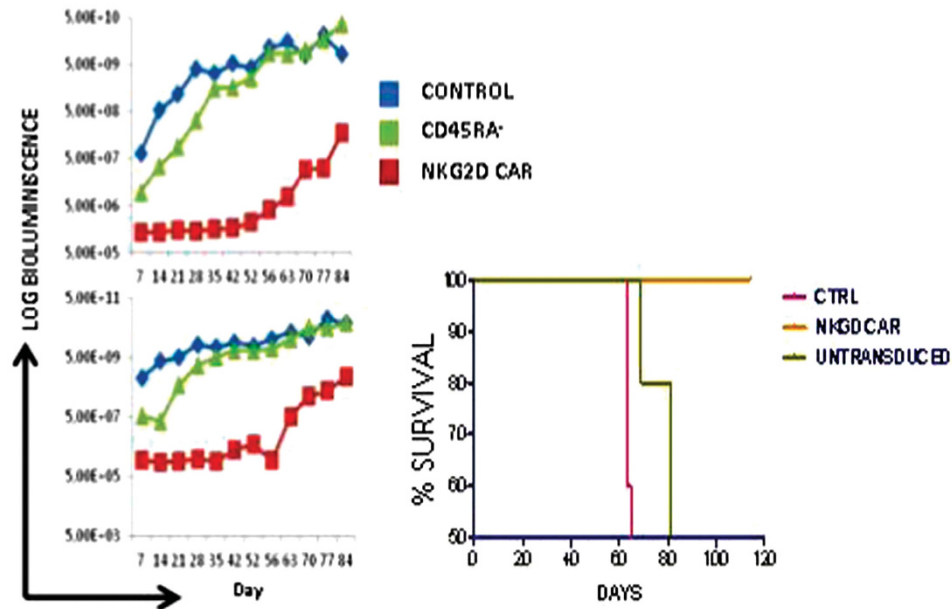
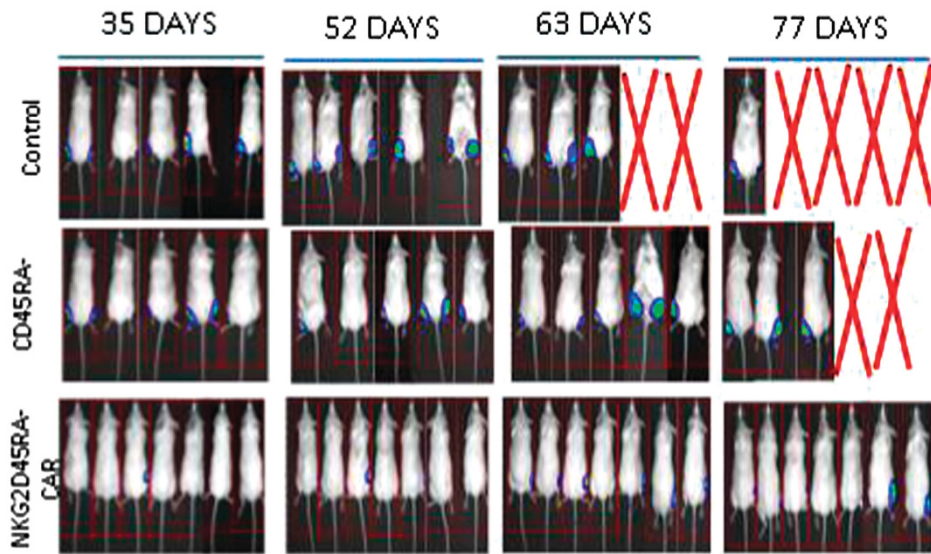
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**Introduction:** Metastatic osteosarcoma has a 5-year survival rate of less than 20%. Current therapies, consistent in radical

[0098]

**NKG2D CAR 45RA- T cells delay osteosarcoma tumor growth and prolong survival**



surgery and neo-adjuvant chemotherapy, remain ineffective. One of the main NK cell activating receptors is NK cell group 2D (NKG2D). NKG2D receptor recognizes human MICA/ULBP1-6 ligands. These NKG2D ligands are expressed in osteosarcoma cells and constitute suitable targets for immunotherapy.

**Material (or patients) and methods:** Peripheral blood mononuclear cells from healthy donors were labeled with CD45RA microbeads and depleted using CliniMACS device. The HL20i4r-MNDantiCD19bbz lentiviral vector were derived from the clinical vector CL20i4r-EF1a-hgcOPT27 but contained the extracellular domain of NKG2D, the hinge region of CD8a and the signaling domains of 4-1BB and CD3-z. The cassette was driven by MND promoter. Viral supernatant was produced by transient transfection of HEK293T cells with the vector genome plasmid and lentiviral packaging helper plasmids pCAGG-HIVgpcp, pCAGG-VSVG and pCAG4-RTR2. The *in vitro* cytotoxicity of 45RA- T cells against 531MII cells was evaluated by performing conventional 4-hour europium-TDA release assays. For the *in vivo* orthotopic model, 531MII YFP-luc cells were used as target in NOD/scid IL2r<sup>gnull</sup> mice.

**Results:** Lentiviral transduction of NKG2D-4-1BB-CD3z markedly increased NKG2D surface expression in CD45RA- cells, which became consistently more cytotoxic than untransduced cells against osteosarcoma cell lines. NKG2D-4-1BB-CD3z expressing T cells had considerable antitumor activity in a mouse model of osteosarcoma, whereas untransduced T cells were ineffective.

**Conclusion:** Our results demonstrate NKG2D-4-1BB-CD3z CAR redirected 45RA negative T cells target NKG2DL expressing osteosarcoma cells both *in vivo* and *in vitro* and could be a promising immunotherapeutic approach for osteosarcoma patients.

**References:** 1. Fernandez L et al. Activated and expanded natural killer cells target osteosarcoma tumor initiating cells in an NKG2D-NKG2DL dependent manner. Cancer letters 2015.

2. Coudert JD et al. The role of the NKG2D receptor for tumor immunity. Semin Cancer Biol. 2006.

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**Disclosure of Interest:** None declared.

#### O099

##### High-dose chemotherapy with auto- or allo-HSCT in high-risk Ewing sarcoma family tumors, a single center experience

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**Introduction:** Ewing sarcoma family tumors (ESFT) are second most common bone tumors in children and young adults. Although aggressive local control combination with systemic therapy allows achieving good results in localized cases, prognosis in patients with primary disseminated disease or large inoperable tumor is still dismal. There is currently no agreement on the role or indications for high-dose chemotherapy (HDCT) with auto-HSCT in ESFT patients due to the lack of randomized trials data. Allo-HSCT is considered an experimental option, as there is only scarce data on graft-versus-ESFT effect. There is also no common agreement on risk stratification.

**Material (or patients) and methods:** A total of 69 high-risk patients with ESFT, median age of 14 (2-37 years), received treatment in 2008-2015. All patients had high-risk disease: primary disseminated tumor ( $n=30$ ), large inoperable primary tumor ( $n=26$ ), chemosensitive relapse ( $n=13$ ). Additional risk stratification was based on score system proposed by Ladenstein R et al for primary-disseminated ESFT. Most

patients received HDCT with auto-HSCT ( $n=56$ ) or allo-HSCT ( $n=6$ ), from haploidentical ( $n=3$ ) or matched unrelated ( $n=3$ ) donors, in 8 patients maintenance therapy was performed. At the time of transplant, 26 (38%) of patients were in CR, 26 (38%) had PR and 4 (14%) had SD. The median follow-up is currently 36 (range 8-99) months. All allo-HSCT recipients received fludarabine-based non-myeloablative conditioning regimens. GVHD prophylaxis consisted of calcineurin inhibitors, MMF and ATG.

**Results:** All maintenance therapy recipients relapsed, 2 of them were salvaged with 2<sup>nd</sup>-line therapy and high-dose consolidation. The results in auto-HSCT group are better ( $P=0.08$ ) with 5-year OS and DFS of 52% and 42%, accordingly. The most important risk factors were number of bone metastases ( $P<0.01$ ), lungs ( $P=0.02$ ) or bone marrow ( $P=0.01$ ) involvement, and induction therapy response ( $P<0.01$ ). Multifactor analysis by Cox regression method revealed additional risk factors: initial tumor volume (HR 3.9,  $P=0.04$ ) and radiation therapy dose (HR 2.9,  $P=0.03$ ). Therefore, the score system proposed by Ladenstein et al allows an adequate risk stratification ( $P<0.01$ ). Although all allo-HSCT recipients died from therapy-related complications ( $n=2$ ) or disease progression ( $n=4$ ), in 5 cases a good response was observed with median time to progression of 6.5 months.

**Conclusion:** Although this trial was not designed to compare high-dose consolidation with maintenance therapy, there is some indirect evidence in favor of intensive approach. The risk stratification score system could possibly be used to determine indications for high-dose consolidation, although further study is needed. Allo-HSCT has very limited effect as salvage therapy, while due to some evidence of graft-vs-ESFT effect,

it may still be effective in some limited patients populations.

**References:** Ladenstein R, Pötschger U, Le Deley MC, et al. Primary disseminated multifocal Ewing sarcoma: results of the Euro-EWING 99 trial. // J Clin Oncol. – 2010. – Vol. 28. – N 20. – P. 3284-91.

**Disclosure of Interest:** None declared.

#### O100

##### Generation of T lymphocytes genetically modified to express third generation GD2-specific chimeric antigen receptor (CAR) with CD28/4.1BB costimulation to improve anti-tumor efficacy of adoptive T cell therapy for patients with neuroblastoma (NB)

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**Introduction:** Adoptive transfer of T cells genetically engineered to express CAR has shown to have therapeutic success in the treatment of B-cell malignancies and solid tumors. Persistence of GD2-specific CAR (GD2-CAR) in patients with relapsed NB was directly associated to a better outcome in the treated patients<sup>1</sup>. However, only a negligible number of NB patients showed a persistence of CAR T cells until 6 weeks after T cell infusion. Recently, it was reported that second generation CAR incorporating 4-1BB costimulation prevents *in vivo* T cell exhaustion<sup>2</sup>. Thus, we test the anti-tumor activity of polyclonal T cells genetically modified with third generation GD2-CAR incorporating the costimulatory molecules CD28 and 4.1BB, in frame with a safety switch, i.e. inducible Caspase 9 (iC9).

**Material (or patients) and methods:** ScFv specific for GD2 was cloned in frame with CD28, 4.1bb and CD3- $\zeta$  (CAR.GD2). Moreover, we added in frame through the 2A sequence the suicide gene iC9. Then, activated T cells were retrovirally transduced and analyzed 5 days after transduction by FACS,

using an anti-idiotype antibody. Cr<sup>51</sup> assays and *in vitro* co-cultures were carried out to evaluate CAR-T cell anti-tumor effect vs GD2+ NB cells. iC9 activation was evaluated by AnnexinV/7AAD staining after exposure to AP1903. To assess the expansion, persistence, and antitumor effect of transgenic CAR.GD2 T cells *in vivo*, we used a NSG mouse model engrafted with luciferase-positive GD2+ human NB cell lines.

**Results:** Flow cytometric analysis of CTLs stained with anti-14. G2a idiotype specific antibody identified chimeric receptors on 40% to 85% of the CTLs (mean, 63%). Chimeric receptor expression was maintained over the entire period of culture (up to 45 days) without any apparent down-regulation. We observed 65% ± 31% of specific cytotoxic activity of CAR.GD2 T cells toward GD2+ NB SH-SY-5Y cells (effector/target ratio 40:1), but not against derived GD2- SH-SY-5Y clone (15 ± 5%). Moreover, 5 day co-culture of CAR.GD2 T cells with GD2+ NB SH-SY-5Y cells (ratio 1:1) showed 1.3 ± 2% of residual tumor cells, whereas 35 ± 18% of GD2- NB cells survived in culture with transduced T cells. Addition of AP1903, the dimerizing agent activating iC9, eliminated CAR.GD2 T-cell activity and induced apoptosis in more than 90% of the transduced cells. The tumor bioluminescence of mice engrafted *i.p.* with SH-SY-5Y cells rapidly increased in recipients of control NT T-cells (rising from 4.6 × 10<sup>8</sup> ± 3.99 × 10<sup>8</sup> to 4.3 × 10<sup>10</sup> ± 8.7 × 10<sup>9</sup> by day 60), while CAR.GD2 T cells significantly controlled tumor expansion so that signal rose from 1.6 × 10<sup>8</sup> ± 9 × 10<sup>7</sup> to only 2.01 × 10<sup>8</sup> ± 3 × 10<sup>8</sup> by day 38 (*P* < 0.0001).

**Conclusion:** Strong and significant cytotoxicity of CAR.GD2 T cells was observed against GD2+ NB cells both *in vitro* and *in vivo* model, demonstrating the potential clinical utility of the retargeted effector cells in the context of solid tumor. In particular, *in vivo* experiments performed over an extended period of time (60 days after T-cell infusion) showed a particular long-lasting T-cell persistence of polyclonal human T-cells gene modified with a third generation CAR vector that include 4.1bb costimulatory molecule.

**References:** 1)Blood. 2011 Dec 1;118(23):6050-6.

2)Nat Med. 2015 Jun;21(6):581-90.

**Disclosure of Interest:** B. De Angelis: None declared, I. Caruana: None declared, D. Orlando: None declared, I. Boffa: None declared, M. Guercio: None declared, M. Sinibaldi: None declared, V. Polito: None declared, D. Pagliara: None declared, A. Moseley Employee of: Bellicum Pharmaceuticals, Inc, M. K. Brenner: None declared, F. Locatelli: None declared, C. Quintarelli: None declared.

## O101

### Lack of benefits of additional chemotherapy after complete remission prior to hematopoietic stem cell transplantation in patients with acute myeloid leukemia: Seattle experience

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**Introduction:** Here, we aimed to examine the effect of post-complete remission (CR; CRp [CR with platelet count < 100,000/mcl] or CRi [CR with absolute neutrophil count < 1,000/mcl] consolidation treatments on the probability of receiving hematopoietic stem cell transplantation (HCT), on having minimal residual disease (MRD) before HCT, and on transplant outcomes in patients with acute myeloid leukemia (AML).

**Material (or patients) and methods:** We examined 383 patients with newly diagnosed AML who were consecutively treated at Seattle cancer care alliance/Fred Hutchinson Cancer Research Center from our database from January 1, 2008, to

Cumulative incidence of transplant by # consolidation courses

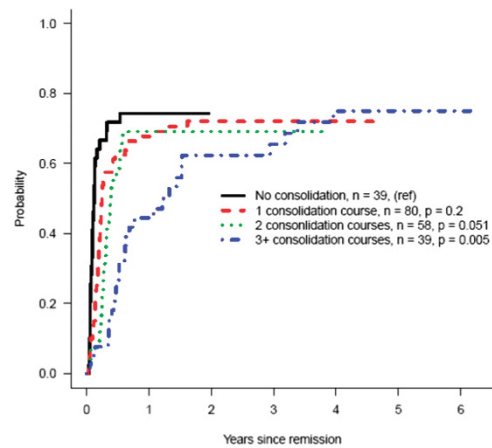


Figure 1: Cumulative incidence of transplant

December 31, 2014. We excluded patients who did not achieve CR, have favourable risk, aged > 70 years, were lost follow-up, or decided not to receive HCT.

**Results:** A total of 215 newly diagnosed AML patients met the inclusion and exclusion criteria and were examined. Patients who received ≥ 1 consolidation treatment and with poor performance status (ECOG > 2) were likely to have a delayed time to transplant. Patients who achieved Cri/CRp with or without MRD at time of the initial response had a delayed time to planned HCT, in comparison to patients with CR. Only 5 of 24 patients (20%) who had CR-MRD after induction and received HCT eventually achieved CR at time of transplant (1 with 1 course, 2 with 2 courses, 2 with ≥ 3 courses of consolidation therapy). CR-MRD persisted in 19 patients (80%), and progression was noted in 1 patient. The administration of > 1 consolidation treatment did not reduce the pre-HCT relapse rate compared to no consolidation treatment.

Table 1. Competing risks regression model for time to relapse from date of response, n = 215

Covariate	HR	95% CI	P-value
3+ consolidation courses (ref=No consolidation)	1.98	(0.94, 4.2)	0.074
CR-MRD (ref=CR)	3.57	(2.02, 6.31)	< 0.001
CRp/CRi (ref=CR)	3.22	(1.63, 6.34)	< 0.001
CRp/CRi-MRD (ref=CR)	4.42	(1.68, 11.67)	0.0027
Low intensity TX (ref=High intensity TX)	0.3	(0.13, 0.7)	0.0054

**Conclusion:** Consolidation therapy is not beneficial for patients scheduled to undergo HCT and prolongs the time to HCT; however, it does not affect the likelihood of receiving HCT but doesn't decrease the relapse rate too, probably because it is inefficient in resolving MRD. Moreover, the presence of CRp/i with MRD prolongs the time to transplant, primarily due to relapses. We believe that patients with intermediate- or poor-risk AML who are indicated for HCT should not receive additional consolidation treatments (especially > 2) as it does not yield better overall survival or leukemia-free survival, does not appear to decrease MRD before HCT, and prolongs the time to HCT, which may be partly related to the complications arising from toxicities and infections.

**Disclosure of Interest:** None declared.

## O102

### DNMT3A mutation in AML patients after allogeneic stem cell transplantation

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**Introduction:** DNMT3A mutation is one of the most frequent somatic mutations in acute myeloid leukemia (AML). Recently discovered persistence of DNMT3A mutations in complete remission (CR) after standard therapy indicates the presence of mutation in early pre-leukemic stem cells.

**Material (or patients) and methods:** Using quantitative PCR, we analyzed DNMT3A mutations in follow up samples obtained from 30 AML patients before and after allogeneic stem cell transplantation (alloSCT). In addition, we examined NPM1, FLT3, IDH1, and IDH2 mutations in diagnostic and follow up samples and donor chimerism after alloSCT. Using well-established markers of molecular remission (molCR) and donor engraftment, we monitored DNMT3A stability during CR.

**Results:** Persistence of DNMT3A mutations in CR after standard therapy was found in all patients. However, in CR after alloSCT in patients with complete donor chimerism, we have no discovered DNMT3A mutation. This data suggests the removal of leukemic stem cells after alloSCT and indicate the importance of alloSCT for high risk AML patients. In relapse of leukemia, all samples showed an increasing of DNMT3A mutated alleles. The loss of correlation between DNMT3A and others mutations in CR after standard chemotherapy could be explained by the presence of mutations in different leukemic clones.

**Conclusion:** We conclude that quantitative detection of DNMT3A mutations at different time points of AML disease could provide additional prognostic information and could explain the role of mutations in development and progression of AML.

**Disclosure of Interest:** None declared.

## O103

### Allogeneic stem cell transplantation in adult patients with acute myeloid leukemia and 17p abnormalities in first complete remission: a study from the Acute Leukemia Working Party (ALWP) of the European society of Blood and Marrow Transplantation (EBMT)

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<sup>10</sup>Hematology, Erasmus Medical Center, Rotterdam, Netherlands,

<sup>11</sup>Hematology, CHU Bordeaux, Bordeaux, France, <sup>12</sup>Hematology,

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**Introduction:** Acute myeloid leukemia (AML) with 17p abnormalities (abn(17p)), usually affecting TP53 locus, carries a very poor prognosis due to high refractoriness to conventional chemotherapy, with long-term survival of less than 5%. Allogeneic stem cell transplantation (SCT) appears as the only potential curative option in high-risk AML. To specifically address outcomes after SCT in patients with

abn(17p), we retrospectively analysed data from the EBMT registry.

**Material (or patients) and methods:** De novo or secondary AML with abnormal karyotype transplanted between 2000 and 2013 have been allocated. From a dataset of 5495 patients with AML undergoing SCT, we included only those patients for whom data were sufficient to confirm the presence of abn(17p) resulting in a loss or a disruption of the TP53 locus.

**Results:** One hundred thirty-nine patients have been selected including 125 patients (90%) in first remission (CR1) and 14 (10%) in second remission. For further analysis, we focused on the 125 patients in CR1. Median age was 54 (range, 18-69) year-old and the median follow-up was 21 (range, 3-146) months. Eighty-five percent of the patients had a de novo AML, while 15% had a secondary AML. Abn(17p) was associated to a monosomal karyotype in 83% of patients, complex karyotype in 91%, monosomy 5 or 5q deletion (-5/5q-) in 55%, monosomy 7 (-7) in 39% and both -5/5q and -7 in 27%, respectively. Median time from diagnosis to CR1 was 57 (range, 18-170) days. Fifty-one (41%) of the patients received a myeloablative conditioning regimen and 73 (59%) had a reduced-intensity conditioning regimen. The vast majority of patients (70%) had a karnofsky performance status of more than 90% at the time of SCT. The 2-year overall survival (OS) and leukemia-free survival (LFS) were 28% and 24%, respectively. The 2-year non-relapse mortality (NRM) was 15%, and 2-yr relapse incidence (RI) was 61%. The cumulative incidence of grade II to IV acute graft-versus-host disease (GvHD) was 24% and that of chronic GvHD was 21%.

In multivariate analysis, the presence of a -5/5q- in addition to abn(17p) was significantly and independently associated with worse OS, LFS and higher RI. Age and donor type did not correlate with outcome. Conditioning intensity was not statistically associated with OS, LFS and NRM when adjusted for patients' age.

**Conclusion:** In contrast to the dismal prognosis reported for AML patients harboring abn(17p) undergoing conventional chemotherapy, allogeneic SCT provides long-term responses in about 25% of a selected group of patients harboring this cytogenetic abnormality at diagnosis and transplanted in CR1. Post and pre transplant adapted therapy may further improve results.

**Disclosure of Interest:** None declared.

## O104

### WT1 and Multiparameter Flow Cytometry MRD status after consolidation significantly predict early relapse in a cohort of 175 Acute Myeloid Leukemia patients

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**Introduction:** Post induction and consolidation minimal residual disease (MRD) might be an independent predictor of AML outcome.

**Material (or patients) and methods:** We evaluated post induction and consolidation bone marrow MRD in 175 AML patients (median age: 57 years, range: 17-89) with 15 months median follow-up (range 1-107). We analysed abnormal leukemia immunophenotype (ALIP) by multi-parameter flow cytometry (MPFC) and WT1 by RT-PCR as described by Buccisano et al and Cilloni et al. Molecular cytogenetic risk was available in 150 patients. We analysed the overall and 1 year Cumulative Incidence of Relapse (CIR), adjusted according to the MRD status, patients and disease characteristics.

**Results:** WT1 was +ve in 129/165 patients (78%) at diagnosis (median 1,136; range: 0-268,784), in 26/113 (23%) post induction (median 21.15; range: 0.4-134,633) and in 13/103

(12.6%) post consolidation (median 21.9; range: 0.4-45,358). MPFC MRD was +ve in 57/100 (57%) patients after induction and in 34/78 (43.6%) after consolidation. 126/151 patients achieved CR, 54 relapsed at a median of 8 months (1-52 months) with 52.2% 5 yr CIR, and 30% 1 yr CIR (N: 39). Patients who underwent chemotherapy (82), Autologous (17) and Allogeneic Transplant (76) as post consolidation treatments had 74.6%, 37.3% and 37.6% 5 yr CIR respectively. NPM+FLT3 ITD- patients had 54.3% 5 yr CIR, compared to 46.7% in NPM-FLT3 ITD-, 35.4% in NPM+FLT3 ITD+ and 76.7% in NPM-FLT3 ITD+ patients. We identified, after induction, a very poor setting of patients with double positive WT1 and MPFC MRD (double positive MRD) with 60% and 58.8% 5 yr and 1 yr CIR respectively; an intermediate group with discordant WT1 and MPFC MRD results (discordant MRD) with 52.7% 5 yr CIR and 38.8% 1 yr CIR; a very good setting with negative WT1 and MPFC values (double negative) with 33.8% 5 yr CIR and 12.1% 1 yr CIR ( $P < 0.0001$ ). Post consolidation MRD analysis showed a 100% 5 yr CIR in patients with double positive MRD vs 65.4% and 24.1% in discordant and double negative patients respectively ( $P < 0.0001$ ). 1 yr CIR was 81.2% in double positive post consolidation MRD, 30.2% in discordant MRD, 12.1% in double negative MRD ( $P < 0.0001$ ) (Figure 1). A multivariate analysis of factors predicting 1 yr CIR confirmed the significant role of post consolidation MRD with 6.98 RR in discordant positive MRD and 12.18 RR in double positive MRD in comparison with double negative patients ( $P = 0.004$ ). The 1 yr CIR analysis also confirmed the predictive role of NPM and FLT3 status with a 0.28 Relative Risk (RR) in NPM-FLT3 ITD- patients, 5.87 RR in FLT3 ITD+ patients compared to NPM+FLT3 ITD- patients ( $P = 0.002$ ). A 5 yr CIR multivariate analysis showed similar results.

**Conclusion:** Post consolidation MRD is a reliable predictor of early relapse regardless of the kind of the following treatment, identifying an extremely poor setting of patients who should be enrolled in an experimental protocol, possibly including MRD driven retreatment.

**Disclosure of Interest:** None declared.

#### O105

##### Prognostic impact of distinct combinations of molecular mutations in AML patients after allogeneic stem cell transplantation

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**Introduction:** To analyze the prognostic impact of distinct combinations of molecular aberrations on outcome after allogeneic stem cell transplantation (alloSCT) for acute myeloid leukemia (AML), a retrospective analysis on 204 adults undergoing related (61) and unrelated (143) alloSCT was performed.

**Material (or patients) and methods:** The mean patient's age was 51 (16-75) years. One hundred fifteen patients (57%) were treated with reduced intensity conditioning. Eighty percent of patients were transplanted in CR (1st or 2nd) and in 20% of patients, alloSCT were performed in relapse of disease. Patients were grouped according to presence of *NPM1*, *FLT3-ITD*, *FLT3-D835*, *DNMT3A*, *IDH1*, and *IDH2* mutations. The influence of aberrant genotypes on relapse, leukemia-free survival (LFS) and overall survival (OS) after alloSCT were analyzed.

**Results:** The presence of *NPM1* mutations were associated with low relapse rate ( $P = .02$ ) and significant better LFS, particularly in cases of single mutation. From 52 *NPM1*<sup>mut</sup> patients, 30 patients with additional *FLT3* mutations was characterized by the inferior LFS as compared with others combination of mutations ( $P = .05$ ). Among 52 *FLT3*<sup>wt</sup> patients, the favorable LFS was seen in patients with *NPM1* mutation and poor outcome was estimated in those with *DNMT3A* mutation ( $P = .03$ ). *IDH1* und *IDH2* mutations less than others

were associated with *FLT3* mutations in our cohort of AML patients. In this group, the lower relapse rate after alloSCT was found ( $P = .0001$ ). The most of *DNMT3A*<sup>mut</sup> patients were associated with *FLT3* and *NPM1* mutations (63%). Patients with *DNMT3A*<sup>mut</sup>/*FLT3*<sup>mut</sup>/*NPM1*<sup>wt</sup> have demonstrated more inferior LFS than *DNMT3A*<sup>mut</sup>/*FLT3*<sup>wt</sup>/*NPM1*<sup>mut</sup>. Among 64 patients with single mutations, the favorable outcome after alloSCT were found in *NPM1*<sup>mut</sup> group and the poor LFS and OS after alloSCT were seen in *FLT3*<sup>mut</sup> and *DNMT3A*<sup>mut</sup> patients ( $P = .01$ ).

**Conclusion:** These results suggest that *DNMT3A*<sup>mut</sup>, particularly when accompanied by *FLT3*-ITD<sup>mut</sup>, is a significant prognostic factor for inferior survival outcome, even after alloSCT.

**Disclosure of Interest:** None declared.

#### O106

##### Five-Year Outcomes of High-Dose Immunosuppressive Therapy and Autologous Hematopoietic Cell Transplantation for Severe Relapsing-Remitting Multiple Sclerosis

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**Introduction:** Most patients with relapsing-remitting multiple sclerosis (RRMS) do not achieve a sustained remission after disease-modifying therapy.

**Material (or patients) and methods:** A phase II clinical trial of high-dose immunosuppressive therapy (HDIT; BCNU, etoposide, ara-C, melphalan and antithymocyte globulin) and autologous hematopoietic cell transplantation (HCT) was conducted in patients with highly active RRMS who had failed conventional therapy to assess the rate of sustained remission. Eligibility required an EDSS of **3.0** (moderate disability, fully ambulatory) to **5.5** (severe disability, ambulatory only 100 meters without aids) and  $\geq 2$  relapses on therapy in previous 18 months. A primary endpoint event or treatment-failure was defined as death or evidence of MS disease activity including any of: 1) relapse 2) new MRI lesions or 3) disability increase  $> 0.5$  EDSS points during 5 years post-transplant. Adverse events (AE) were recorded according to NCI-CTCAE v3.0. In addition, the Multiple Sclerosis Impact Scale (MSIS-29), a patient-based quality of life measure, and the Multiple Sclerosis Functional Composite (MSFC), a three-part standardized assessment instrument, were used to assess MS disease activity.

**Results:** 25 patients with median age 37(26-52) years were treated with G-CSF and prednisone to mobilize the autograft. The autograft was CD34-selected (Baxter, Isolex). 24 patients received HDIT/HCT according to protocol. Median follow-up was 62 months (min 12, max 72). In the first 3 years after HDIT, there were 121 grade 3 and 93 grade 4 AE, mostly hematological and gastrointestinal. Between 3 and 5 years after transplant, there were 15 grade 3 and 0 grade 4 AE. 3 deaths occurred on the study, all after subjects had met another MS disease activity or disability component of the composite endpoint. One patient experienced progressive loss of neurological function and death at 32 months. Also, two patients died  $> 3$  years post-transplant. None of the deaths were related to study treatment.

At 5 years, the probability of event-free survival according to the primary endpoint was 69.2% (90% CI: 50.2% > 82.1%); progression-free and relapse-free survival were 90.9% (90% CI: 73.7% > 97.1%) and 86.3% (90% CI: 68.7% > 94.5%), respectively, and probability of freedom from disease activity detected by brain MRI was 88.2% (90% CI: 67% > 96.2%). As reported in our interim analysis of three-year outcomes [1], in which 7 out of 24 patients who received HCT met primary endpoint, no further events occurred by the close of 5 years. Further, MS disease burden as measured by T2 weighted lesion volume on MRI was significantly reduced by 6 months as compared to baseline and was sustained for 5 years (median change: -1.208 ml;  $P < 0.001$ ). T1 lesion volume was increased at 5 years (median change: 0.094 ml;  $P = 0.041$ ). While both the MSIS-29 and the MSFC showed improvement at Year 5 with median difference from baseline of -8.5 ( $P = 0.091$ ) and 0.11 ( $P = 0.303$ ), respectively, changes were not statistically significant.

**Conclusion:** HDIT/HCT for highly active RRMS induced a high rate of remission of MS disease activity which was sustained at 5 years, without maintenance therapy. Most treatment-related AE were as expected, consistent with the transplant regimen.

**References:** 1. Nash RA, et al. *JAMA Neurol.* 2015;72(2):159-169.

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#### O107

##### Functional evaluation of systemic sclerosis patients after autologous hematopoietic stem cell transplantation

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**Introduction:** Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by skin thickening and visceral involvement, leading to impairment of physical function, daily life activities and quality of life. Severe cases usually have poor prognosis, despite conventional immunosuppressive treatment. Autologous hematopoietic stem cell transplantation (AHSCT) has been investigated as treatment for patients with severe SSc, improving skin and at least stabilizing lung involvement. There are no reports in the literature that address the influence of AHSCT on the functional capacity of SSc patients. The present study aimed to evaluate the impact of AHSCT on skin involvement, functional capacity and quality of life (QoL) of SSc patients, and to compare these results with those from SSc patients under conventional immunosuppressive treatment.

**Material (or patients) and methods:** In this prospective, longitudinal study, SSc patients treated with AHSCT (transplant

group) and patients under conventional immunosuppressive treatment (control group) were evaluated before, and six months after treatment. The evaluations included respiratory muscle strength test (maximal inspiratory pressure – MIP and maximal expiratory pressure-MEP), hand function assessments (hand-grip strength, finger-to-palm distance-FTP, COCHIN questionnaire), six-minute walk test (6MWT), modified Rodnan's skin score (mRSS) and quality of life questionnaire (SF-36). Results underwent statistical analyses and significance levels were established at  $P < 0.05$ .

**Results:** Eleven SSc patients were included in the transplant group and 11 in the control group. At 6 months after AHSCT, transplanted patients presented improvement of MIP ( $P = 0.0064$ ), MEP ( $P = 0.0039$ ), 6MWT walking distance ( $P = 0.0078$ ), right hand FTP distance ( $P = 0.0304$ ), left ( $P = 0.0016$ ) and right ( $P = 0.0007$ ) hand-grip strength, COCHIN ( $P = 0.0011$ ), mRSS ( $P = 0.0005$ ), and of physical component score of SF-36 ( $P = 0.0018$ ). No significant differences were detected in the remaining evaluations. In the control group, no significant changes were observed 6 months after treatment at any of the evaluations. When comparing transplant and control groups, we observed statistically significant differences in MIP ( $P = 0.0008$ ), MEP ( $P = 0.0084$ ), 6MWT walking distance ( $P = 0.0067$ ), right and left hand-grip strength ( $P = 0.0168$ ), COCHIN ( $P = 0.0052$ ), mRSS ( $P = 0.0031$ ), and physical component score of SF-36 ( $P = 0.0121$ ).

**Conclusion:** AHSCT significantly improved functional capacity, skin involvement and quality of life in SSc patients when compared to baseline and also when compared to the control group. These results can be interpreted as positive outcomes of AHSCT for SSc.

**References:** van Laar et al. *JAMA* 2014;311:2490-8.

Burt et al. *Lancet* 2013;381:1116-24.

**Disclosure of Interest:** None declared.

#### O108

##### Sustained improvements in neurological function following autologous hematopoietic cell transplant in patients with multiple sclerosis utilizing a lympho-depleting non-myeloablative regimen

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**Introduction:** Autologous hematopoietic cell transplant (auto-HCT) for multiple sclerosis (MS) has been performed worldwide since the late 1990s, however, it is still not regarded as standard treatment. We present the results of patients treated in Singapore since 2002 on a Flu/Cy conditioning regimen that is to our knowledge, unique to our centers in its application to MS.

**Material (or patients) and methods:** Eligible patients had either relapsing remitting (RR) or secondary progressive (SP) MS and failed at least 1 previous disease-modifying drug (DMD); failure was defined as at least 1 flare in the preceding 12 months while on treatment in RRMS patients, or ongoing neurological decline in SPMS. Mobilization was with IV cyclophosphamide 2g/m<sup>2</sup> and filgrastim. PBSC were enriched for CD34 by use of the CliniMacs CD34 system (Miltenyi Biotec) before cryopreservation. The conditioning regimen consisted of IV fludarabine 30mg/m<sup>2</sup> from d5 to d-3 and IV cyclophosphamide 50 mg/kg from d-5 to d-2. Neurological progress was assessed by a neurologist using the Kurtzke Expanded Disability Status Scale (EDSS).

**Results:** Between 2002 and 2015, 31 patients underwent transplant; 2 patients were subsequently diagnosed with neuromyelitis optica and excluded from this study. 25 patients (8 male: 17 female) with at least 3 months follow up were considered for this analysis. 19 patients had RRMS and 8 had

SPMS. The median age at transplant was 37.4y (range 20.8y to 56.3y). Neutrophil engraftment (defined as the first day after transplant that absolute neutrophil count exceeded 500/uL) occurred at a median of 10 days (range 8-11d); time to platelet count > 50/uL was 14d (range 10-17). At a median follow up of 12.5m (range 3m to 157m), the event-free survival was 84%. Eight patients experienced febrile neutropenia that responded to antibiotics, with only 1 case of grade 5 severity (described below). Four deaths occurred, of which 3, all with SPMS and baseline EDSS  $\geq 7.5$ , were related to MS progression and death from infection 6 or more months after HCT. One patient, with RRMS, died following severe pseudomonas sepsis and engraftment respiratory syndrome occurring during transplant, eventually succumbing at 3m post-transplant from pneumonia. Serial EDSS is shown in Figure 1 and demonstrates improvement in most patients with RRMS, all of whom have discontinued DMD following transplant. Surviving patients with SPMS have remained stable (no change in EDSS > 1.0) post transplant.

**Conclusion:** The Flu/Cy regimen is well tolerated, with only one case of toxic death in our series since 2002. All patients with RRMS have been able to discontinue DMDs following transplant, with neurological improvement seen in all patients with baseline EDSS < 6.0. SPMS patients were more likely to progress and succumb to complications of worsening MS, particularly when EDSS was > 7.5 pre-HCT. While more DMDs have been approved for treatment in recent years, most require long-term treatment to sustain remission. This supports considering auto-HCT for patients with MS not controlled despite DMDs.

**Disclosure of Interest:** Y. Loh Funding from: Honoraria: Novartis, Gilead, Janssen, Sanofi, Celgene, L. Donato: None declared, W. Hwang: None declared, Y. C. Linn: None declared, Y.T. Goh: None declared, A. Ho: None declared, A. Tauqeer: None declared, A. Seah: None declared, P. Ratnagopal: None declared, N. Dali: None declared, J.J. Lee: None declared, C. Phipps: None declared.

**O109**

**Thymic output after immunoablation and CD34-selected autologous stem cell transplantation generates a new and diverse pool of natural regulatory T cells in systemic lupus erythematosus**

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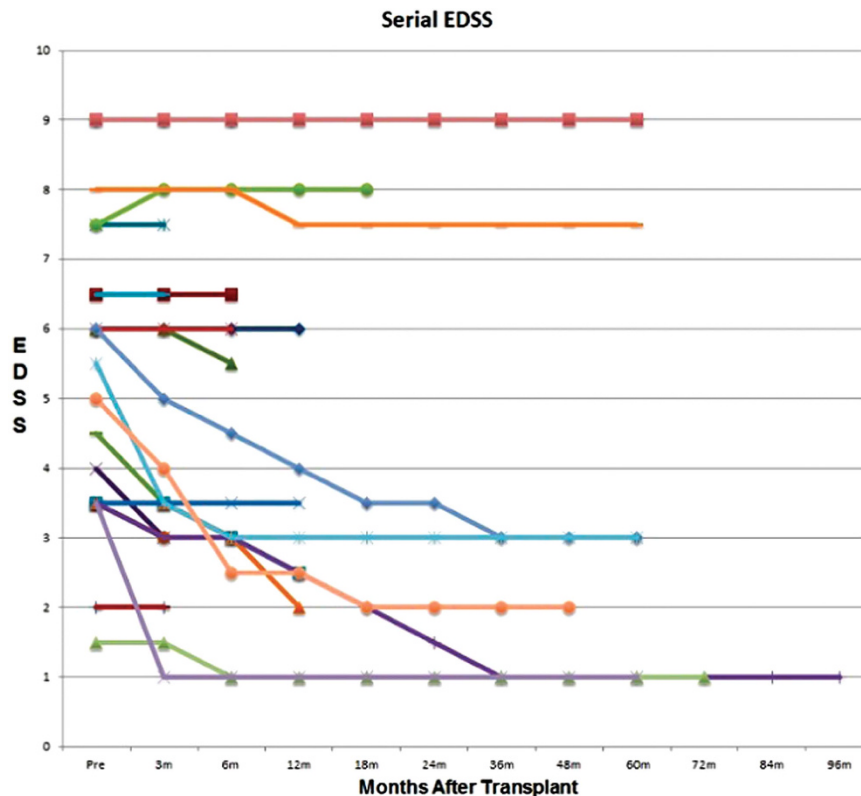
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**Introduction:** Our previous research has provided the evidence that an autoreactive immune system can be "reset" into a healthy, tolerant state by immunoablative treatment to eradicate pathogenic effector cells, followed by transplantation of hematopoietic progenitor cells (HSCT). Here, we investigated the T cell reconstitution in 10 patients with refractory systemic lupus erythematosus (SLE) for up to 15 years after receiving immunoablation and ASCT.

**Material (or patients) and methods:** Since 1998, 10 patients with refractory SLE received a CD34<sup>+</sup>-selected autologous stem cell transplantation after immunoablation with ATG and cyclophosphamide as part of a prospective monocentric phase I/II clinical trial. Their peripheral blood lymphocytes were investigated using multiparametric flow cytometry, including analysis of the TCR-Vbeta repertoire on CD4<sup>+</sup> lymphocytes. Thymic activity was determined measuring absolute counts of peripheral blood CD31<sup>+</sup> thymic naive Th cells. In addition, Tregs were analyzed for their expression of Helios, CD45RA and CD31. Healthy donors and patients after thymectomy for myasthenia gravis served as controls.

**Results:** In the first 6 months post-Tx most CD4<sup>+</sup> T cells presented a CD45RO<sup>+</sup> memory-like phenotype and TCR analysis revealed a highly restricted TCR Vbeta usage resulting from peripheral expansion. Subsequently, an increase in

[0108]



absolute numbers of CD31<sup>+</sup> thymic naïve CD4<sup>+</sup> T cells occurred 6-12 months post-Tx and persisted in significantly higher numbers compared to age-matched controls. Expression levels for Helios – a marker for natural Tregs – in recurring Tregs post-Tx was similar in SLE compared to healthy controls and thymectomized patients (~70%), but their coexpression levels for CD45RA and CD31 was significantly higher (26.4% vs. 20.6% vs. 7.1%). While expanded naturally occurring Helios<sup>+</sup> Tregs in active SLE patients under conventional treatment showed a skewed TCR repertoire, the TCR repertoire of Tregs in responding patients after ASCT was diverse.

**Conclusion:** Our data show that immune reconstitution after ASCT is associated with a profound reconfiguration of the adaptive immune system, i.e. immune reset, characterized by stable thymic reactivation with recurrence and persistence of thymic naïve conventional and regulatory T cells. Thymic output generates a pool of new and diverse CD4<sup>+</sup> T cells. Although Helios is regarded as a marker for naturally occurring Tregs, coexpression levels for CD31 and CD45RA better correlates with thymic output conditions for Tregs.

**Disclosure of Interest:** None declared.

### O110

#### TSC-mTOR Signaling Pathway Regulates the Immunogenicity of Bone Marrow Mesenchymal Stem Cells in Inflammatory Microenvironment

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**Introduction:** Mesenchymal stem cells (MSCs) have been utilized to treat graft-versus-host disease (GVHD) and some autoimmune diseases due to their extensive immunomodulatory activities. However, the immunogenicity of MSCs weakens their immunomodulatory effect especially in inflammatory microenvironment, which could enhance the immunogenicity. Here we address the role of TSC-mTOR signaling pathway on regulation of the immunogenicity of MSCs.

**Material (or patients) and methods:** Bone marrow MSCs were isolated from healthy donors. The immunomodulatory

function of MSCs was determined by inhibiting the proliferation of T cells. After pretreatment with various concentration of rapamycin, HLA-ABC<sup>+</sup>, HLA-DR<sup>+</sup> cell proportion and mean fluorescence intensity (MFI) in normal culture or treated with IFN- $\gamma$  were evaluated by flow cytometry. We used lentivirus-mediated shRNA interference to assess the role of TSC2 in regulation of the immunogenicity of MSCs. Western blotting was used for evaluating the mTOR signaling activities and phosphorylation of Stat1. The mRNA expression of IRF1, CIITA, proteasome subunits and MHC-I assembly related molecular chaperones were detected by real time PCR.

**Results:** We first found that mTOR inhibition by pretreatment with rapamycin significantly enhanced the immunosuppressive function of MSCs (Figure 1A, B). To investigate whether mTOR inhibitor influence the immunogenicity of MSCs, HLA molecules were detected after treating with rapamycin. In normal culture, mTOR inhibition has no effect on the expression of HLA-ABC. The MFI of HLA-ABC was elevated by treating with IFN- $\gamma$  for 3 days. Proteasome subunits LMP2, LMP7, LMP10 and molecular chaperones, including Tapasin, were also upregulated. Rapamycin has no effect on the expression of HLA-ABC in culture treated with IFN- $\gamma$  as well. MSCs were negative for HLA-DR, and its expression was significantly elevated by IFN- $\gamma$ . The phosphorylation of Stat1 and expression of IRF1, CIITA were also upregulated. IFN- $\gamma$  induced HLA-DR expression was not involved with mTOR signaling as indicated by no difference in mTOR substrate activities. However, mTOR inhibition significantly reduced not only HLA-DR<sup>+</sup> cell proportion but also HLA-DR MFI in culture treated with IFN- $\gamma$  3 days or 5 days later (Figure 1C, D). Conversely, reducing the expression of tuberous sclerosis complex 2 (TSC2) using shRNA, which negatively regulates the mTOR, significantly enhanced the HLA-DR expression in culture treated with IFN- $\gamma$ .

**Conclusion:** mTOR inhibition increased the immunomodulatory function of MSCs, and reduced the expression of HLA-DR upregulated by IFN- $\gamma$ , which may provide a new strategy to improve MSC-based immunotherapy in inflammatory microenvironment.

**Disclosure of Interest:** None declared.

### [O110]

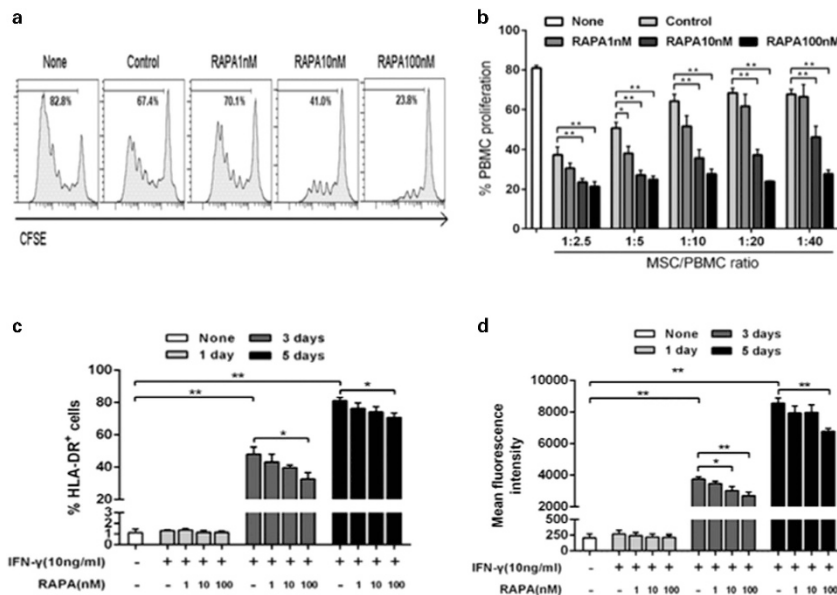


Figure 1: mTOR inhibition regulates the immunomodulatory function and immunogenicity of MSCs.



O111

**Comparison of haplo-identical versus unrelated allogeneic stem cell transplantation for acute myeloid leukemia with active disease: A report of 1578 patients from Acute Leukemia Working Party of EBMT**

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**Introduction:** Primary refractory or relapsed acute myeloid leukemia (R-AML) is associated with dismal prognosis. Allogeneic transplantation (HSCT) in the setting of active disease is the alternative strategy. Recently, our group reported that a matched sibling donor or an unrelated donor (UD) (10/10 and 9/10) did not have any impact on primary refractory AML patient outcomes (Brissot *et al*, ASH 2015). In contrast, time to transplant was a major prognostic factor for leukemia-free survival (LFS).

Unmanipulated grafts are increasingly used in the haplo-setting, and innovative regimens for GvHD prophylaxis have been adopted with encouraging results. Haplo-identical donors (HD) are available for practically almost all AML patients (pts) without any delay for transplant.

A comparison of the outcomes between HD versus UD in HSCT for R-AML is therefore of special interest, and may contribute to guide our practical management of active AML.

**Material (or patients) and methods:** The current study aimed to compare the outcomes of R-AML pts who received HSCT from a HD ( $n=225$ ) versus an UD ( $n=1353$ ).

Patients with R-AML, who underwent an HSCT with a HD or an UD between 2007 and 2014 and reported to the registry of the EBMT ALWP, were included.

The major endpoints were to assess LFS, relapse incidence (RI), non-relapse mortality (NRM), and GVHD and relapse free survival (GRFS) (Ruggeri *et al*, BMT, 2015, *in press*).

**Results:** 119 pts received a transplant from an HD with cyclophosphamide post-transplant (HD-PTCy), and 106 an HD with other types of immunosuppressive treatment (HD-IM) while, 1003 pts received a transplant from matched UD 10/10, and 350 pts from a mismatched UD (9/10). The LFS at 2 years was 21.3% in HD-PTCy, 7.3% for HD-IM, 28.1% for UD 10/10, and 20.9% in UD 9/10 respectively. In multivariate analysis, comparing to the 10/10 UD group, there was no statistically significant difference with the HD-PTCy group (HR=1.03, 95% CI, 0.78-1.36,  $P=0.850$ ) and with the UD 9/10 group (HR=1.16, 95% CI, 0.99-1.36,  $P=0.061$ ), and while LFS was significantly lower in the HD-IM (HR=1.50, 95% CI, 1.18-1.90,  $P=0.001$ ). RI at 2 years did not differ between the 4 pts groups being 50.8% in HD PTCy, 58.3% for HD-IM, 45.8% for UD 10/10, and 51.1% in UD 9/10. In multivariate analysis, 3 predictive factors were associated with a higher RI: HD-IM, patient age, and poor cytogenetics. For NRM, patient age was associated with higher

NRM whereas RIC regimen and KPS  $\geq 90\%$  were protective factors. Finally, HD-PTCy and UD 9/10 had a comparable GRFS to UD 10/10 (HR=1.07, 95% CI, 0.92-1.24,  $P=0.395$ , and HR=0.96, 95% CI, 0.74-1.26,  $P=0.96$ ; respectively). In contrast, HD-IM resulted in a significant lower GRFS (HR=1.38, 95% CI, 1.09-1.73,  $P=0.007$ ). RIC regimen and KPS  $\geq 90\%$  were associated with a better GRFS (HR=0.87, 95% CI, 0.76-0.99,  $P=0.033$ , and HR=0.64, 95% CI, 0.56-0.72,  $P < 10^{-4}$ ; respectively).

**Conclusion:** HSCT may rescue 20% of R-AML patients. Importantly, the outcomes of transplants from UD 9/10 or HD-PTCy were comparable to UD 10/10 whereas HD with immunosuppression as anti GVHD prophylaxis had a significantly lower LFS and GRFS at 2 years. Therefore, HD-PTCy is a valid option for R-AML pts with the advantage of high donor availability and with no delay which is of major importance in the setting of R-AML.

**Disclosure of Interest:** None declared.

O112

**Graft predominance and graft versus leukemia early after double Umbilical Cord Blood Transplantation (dUCBT): a novel role for CD4+ T-cell effector alloreactivity towards mismatched HLA class alleles**

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**Introduction:** Graft predominance after dUCBT was earlier suggested to be CD8+ T-cell mediated, but is still incompletely understood. We recently showed that CD4+ T-cells rapidly expand after dUCBT and early CD4+ T-cell chimerism predicts for graft predominance. Given the frequent HLA class II allele mismatches between the 2 UCB units in dUCBT, we hypothesized that HLA class II-specific CD4+ T-cells from the 'winning' CBU may be responsible for rejection of the 'loser' CBU. In order to test that hypothesis, we evaluated whether 'winning' CD4+ T-cells specifically recognize individual HLA class II allele mismatches, expressed by the rejected graft, and if present, whether such T-cells also recognize recipient leukemic cells.

**Material (or patients) and methods:** Post dUCBT patient T-cells were propagated in-vitro and subsequently evaluated in a co-culture with class II allele transduced HELA cells. T cell specificities, activation and effector markers were assessed by flow cytometry (FCM). Patient selection criteria included single graft predominance and at least 1 class II mismatch between both units.

**Results:** Eleven patients with poor-risk leukemia were studied in depth, receiving 22 UCB units matched at HLA A, B, and DRB1 for 5/6 ( $n=7$ ) or 4/6 ( $n=15$ ) with the recipient. The median number (range) of class II allele mismatches between the 2 UCB units per transplant was 2 (1-6). In total, 33 different class II allele mismatches were tested, including 16 at HLA DR, 7 at DQ, and 10 at DP. Peripheral blood CD3+ T-cell numbers of samples taken at 1-6 months post UCBT were low (median: 0.207; range 0.030-0.699  $\times 10^{-9}/L$ ) with 74% (range, 8-96%) consisting of CD4+ T-cells. In all 11 patients, alloreactive CD4+ T-cells towards one or more mismatched class II alleles were detectable. In total, CD4+ alloreactivity towards 29 out of 33 (88%) mismatches was detected, including 15/16 for DR (94%), 7/7 for DQ (100%), and 7/10 (70%) for DP alleles. Stronger CD4+ T-cell reactivity was observed towards DR and DQ as compared to DP. Limited activity towards matched, control alleles was shown in 2/11 (18%) combinations and in 3/17 (18%) with respect to irrelevant third party alleles. The highest alloreactive responses were observed at 1 month post UCBT. Alloreactive CD4+ T-cells upregulated activation markers CD137, CD134 and PD1 and the effector markers CD107 (degranulation) and Interferon-gamma. Moreover, leukemia cells from recipients exhibiting the same class II mismatch as

the rejected unit, were also recognized by effector CD4+ T-cells.

**Conclusion:** Collectively, these results demonstrate that specific effector alloreactivity by 'winner' CD4+ T-cells directed to multiple class II mismatched alleles was present in all patients, already at 1 month post UCBT. These results suggest that immediate cytotoxicity exerted by CD4+ T-cells from the 'winning' cord represent a novel mechanism of rapid rejection of the 'losing' unit after dUCBT. Furthermore, it is suggested that the low incidence of relapse observed after dUCBT may, at least in part, result from an immunological, graft-versus-leukemia effect exerted by effector CD4+ T-cells, evoked by mismatched class II alleles expressed by the rejected graft. Evaluation of such CD4+ effector alloreactivity towards class II major mismatches after haploidentical transplantation is now warranted.

**Disclosure of Interest:** None declared.

#### O113

##### **Allogeneic Hematopoietic Stem Cell Transplantation from unrelated peripheral blood or bone marrow donors: The impact of HLA matching including HLA-DPB1 allele in a multivariable risk model**

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**Introduction:** We conducted this study to evaluate the impact of HLA matching degree between patient receiving allogeneic HSCT for hematological malignancies and HSC donor including HLA-DPB1, taking into account the other impacting variables in the allo-HSCT settings.

**Material (or patients) and methods:** A total of 235 patients who received allo-HSCT between January 2005 and December 2014 were included, 131 (56%) were males, median age at allo-HSCT was 50 years (range: 18-69). There was 123 (53%) acute leukemia (93 AML, 30 ALL), 24 (10%) MDS, 35 (15%) multiple myeloma, 20 (8%) NHL, 7 (3%) Hodgkin's disease, 10 (4%) myeloproliferative neoplasms, 13 (6%) CML, and 3 (1%) CLL; 119 (51%) patients received MAC and 116 (49%) received RIC. Disease status at allo-HSCT was CR in 144 (61%) patients and less than CR in 91 (39%). HSC donor was 10/10 HLA matched unrelated (MUD) for 162 (69%) (80 PBSC and 93 BM), among them 21 (9%) were matched for HLA-DPB1, 41 (18%) had permissive mismatch (pMM) and 100 (42%) had non-permissive mismatch (npMM); while 73 (31%) had 9/10 HLA mismatched donor MMUD (48 PBSC and 25 BM), among them, 7 (3%) were matched for HLA-DPB1, 12 (5%) had pMM and 54 (23%) had npMM; 110 (47%) were ABO compatible, 58 (24%) had minor incompatibility and 67 (29%) had major incompatibility. For sex mismatching, in 33 (14%) cases, it was female donor to a male patient.

**Results:** After a median follow-up for surviving patients of 29 months (range: 4-108), patients with 10/10 HLA MUD had better overall survival (OS) than those with 9/10 MMUD without considering the HLA-DPB1 matching, with 2 years OS probability of 51% vs 35% respectively ( $P=0.09$ ), which was reflected by a lower NRM at 2 years of 29% vs 42% ( $P=0.07$ ). When considering the HLA-DPB1 matching, we found comparable outcomes in terms of OS and NRM for: 1) 10/10 HLA MUD-DPB1 matched vs 10/10 HLA MUD-DPB1 pMM, 2) 10/10 HLA MUD -npMM DPB1 vs 9/10 HLA MMUD-DPB1 matched, 3) 9/10 HLA MMUD-DPB1 matched vs 9/10 HLA MMUD-DPB1 pMM; all these 3 groups were not significantly different between each other except for a last group which included 9/10 HLA MMUD with npMM-DPB1, this group had worse OS and NRM compared to all others with 2 years rates of 34% vs 49% ( $P=0.05$ ) and 47% vs 29% ( $P=0.04$ ) respectively. In multivariate analysis, patient age (> 50 years), disease status (less than CR), HLA matching (9/10 HLA MUD npMM DPB1) and sex mismatching (female donor to male patient) were

significantly impacting OS and NRM. We included all these variables in a risk score: age < 50 years=0, > 50 years=1; CR=0, less than CR=1; HLA 10/10 (matched on DPB1) or HLA 10/10 with permissive MM on DPB1=0; HLA 10/10 npMM on DPB1 or HLA 9/10 (matched on DPB1 or pMM on DPB1)=1; HLA 9/10 npMM on DPB1=2; for sex matching, female donor to male patient=1, all other=0. The risk score distinguished low risk patients (total score=0), intermediate (total score=1 or 2) and high risk (total score > 2) with 2 years OS and NRM rates of 66%, 52%, 30% ( $P=0.003$ ) and 22%, 29% 48% ( $P=0.004$ ) respectively.

**Conclusion:** MMUD with non-permissive T-cell-epitope mismatch at HLA-DPB1 should be avoided due to increased rates of NRM. The risk score combining HLA matching with age, disease status and sex matching is very helpful for daily clinical practice offering patients better treatment strategy.

**Disclosure of Interest:** None declared.

#### O114

##### **Umbilical Cord Blood Transplantation Supported by Third Party Donor Cells (Haplo-cord HSCT) Compared to Unmanipulated Haploidentical Transplantation with Post-transplant Cyclophosphamide (PTCY-haplo HSCT) in Patients with Acute Myeloid Leukemia**

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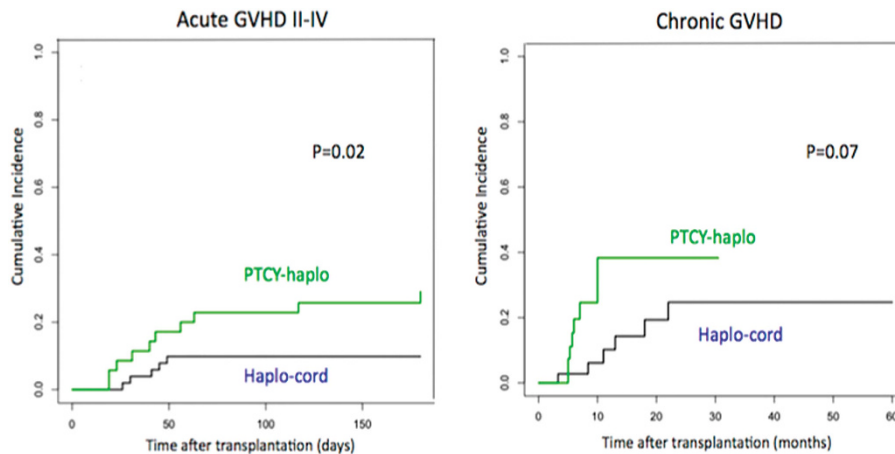
**Introduction:** For patients with acute myeloid leukemia (AML) without an HLA matched donor, the best alternative donor remains yet to be defined. We analyze outcomes of patients with AML who underwent umbilical cord blood or haploidentical HSCT in Spain.

**Material (or patients) and methods:** Between 1999 and 2012, 51 patients underwent single umbilical cord blood myeloablative transplantation supported by CD34+ cells from a HLA-mismatched third party donor (haplo-cord HSCT), and between 2012 and 2014, 36 patients received a myeloablative unmanipulated haploidentical transplantation with post-transplant cyclophosphamide as GVHD prophylaxis (PTCY-haplo HSCT) in GETH centers in Spain.

**Results:** The 30-day cumulative incidence of neutrophil engraftment was 100% in the haplo-cord and 97% in the PTCY-haplo group with a median time to engraftment of 12 and 17 days, respectively ( $P=0.01$ ). The 60-day cumulative incidence of platelet recovery was 82% vs 72%, in a median time of 35 and 29 days, respectively ( $P=0.8$ ). The rate of grade II-IV acute GVHD was significantly higher in the PTCY-haplo HSCT group (9.8% vs 26%,  $P=0.02$ ) and chronic GVHD rates showed a higher tendency in the PTCY-haplo HSCT group (20% vs 30%,  $P=0.07$ ). With a median follow-up of 61 months for the haplo-cord HSCT group and 11 months for the PTCY-haplo HSCT group, OS was 55% and 56% ( $P=0.83$ ), EFS was 45% vs 54% ( $P=0.57$ ), relapse rate was 22% vs 28% ( $P=0.83$ ), and TRM was 22% vs 17% ( $P=0.64$ ), respectively, with no significant differences in outcomes.

**Conclusion:** In this multicentric experience, myeloablative cord blood transplantation supported by third party

[0114]



HLA-mismatched donor and haploidentical transplantation with post-transplant cyclophosphamide offer valid alternatives for patients with AML. Engraftment rates were similar in both groups, significantly faster in the haplo-cord group, as well as survival rates. However, GvHD rates were higher after haploidentical HSCT. Significant differences in follow-up time and period of transplant execution compel to take these results cautiously.

**Disclosure of Interest:** None declared.

O115

**Winning Cord Blood Unit Characteristics Impacting on Outcomes after Double Umbilical Cord Blood Transplantation in Adults with Acute Leukemia: a Collaborative Study of Eurocord and the Acute Leukemia Working Party of EBMT**

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**Introduction:** Double umbilical cord blood transplantation (dUCBT) has been widely used in adults with acute leukemia (AL). Chimerism analysis usually shows that only one cord blood unit (CBU) provides long term hematological reconstitution, but no factor has been yet identified to reliably predict which will be the winning unit (winCBU).

**Material (or patients) and methods:** To determine factors that may predict the winCBU (defined as the CBU representing >50% of the recipient (rcp) hematopoiesis) and the impact of the winCBU characteristics on outcomes after dUCBT, we studied adults with AL who underwent dUCBT as first transplant from 2004 to 2013 reported to Eurocord. We analyzed 347 patients (pts) who engrafted, had available chimerism data and for whom a winCBU was identified.

**Results:** Among 347 pts, 323 had full donor and 24 had mixed chimerism (>5% of donor cells). Diagnosis was ALL for 35% and AML for 65%. At dUCBT 45% were in first complete remission (CR), 44% in second CR and 11% had more advanced disease. Pts median age was 40 years. Median time from diagnosis to dUCBT was 12 months. For winCBUs, median number of TNC and CD34 cells at cryopreservation was 2.5x10<sup>7</sup>/Kg and 1x10<sup>5</sup>/Kg, respectively. Only 4% of the winCBUs were 6/6 HLA-matched to the rcp, 37% were 5/6, 55% were 4/6 and 4% were 3/6 HLA-matched. WinCBUs median age was 3.4 years (0.2-14), 54% of the winCBUs were gender matched and 38% were ABO compatible with the rcp. Median follow-up for survivors was 35 months. In this population, cumulative incidence (CI) of 100-day acute GvHD grade II-IV was 41%. The 3y-CI of chronic GvHD, relapse (RI) and transplant related mortality (TRM) were 41%, 27% and 24%, respectively. At 3y, leukemia free survival (LFS) was 49% and overall survival (OS) was 54%. In MVA, no significant factors predicting the winCBU were identified after adjusting for HLA and gender matching, type of HLA MMs, TNC and CD34+cells, ABO compatibility, CBU age and inter unit features. Pts with 6/6 or 5/6 HLA-matched winCBUs had a 3y-LFS of 56% compared to 44% for those with 4/6 (P=0.03), while OS was 62% versus 49% (P=0.01), respectively. Acute GvHD was increased in pts receiving a 4/6 HLA-matched winCBU (46% versus 35% for those 6/6 or 5/6, P=0.04). In MVA, 4/6 HLA-matched winCBU was associated with lower LFS (HR 1.5, P=0.03) and OS (HR 1.5, P=0.03), and with increased TRM (HR 1.9, P=0.03) and acute GvHD (HR 1.7, P=0.01). Older winCBUs (>3.4y) were associated with higher acute GvHD (48% versus 35%; HR 1.6, P=0.02). ATG use and advanced disease were associated with poor outcomes.

**Conclusion:** We demonstrated that specific winCBU characteristics have an impact on dUCBT outcomes. In particular, 4/6 HLA matched winCBU is associated with decreased LFS and OS, and with higher NRM and aGvHD. No factors predicting CBU predominance were identified.

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with: TEVA, SUNESIS- Honoraria, S. Vigouroux: None declared, C. F. Craddock: None declared, C. Kenzey: None declared, M. Mohy Funding from: Riemsers, E. Gluckman: None declared, A. Nagler Conflict with: Biokine LTD, V. Rocha: None declared.

#### O116

### Allogeneic Transplantation for Relapsed / Refractory (R/R) Follicular Lymphoma (FL). A Joint Study Between the European Society for Blood and Marrow Transplantation (EBMT) and the Center for International Blood and Marrow Transplant Research (CIBMTR)

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**Introduction:** The definitive management of R/R FL remains controversial. Nevertheless, allogeneic hematopoietic stem cell transplantation (allo-SCT) remains the sole curative therapy for FL. Herein, we report the long term outcome of the largest sample of allo-SCT for FL ever studied as well as the identification of patient and disease related factors that were significantly associated with treatment failure.

**Material (or patients) and methods:** Eligible were adult patients with R/R FL having received a first allo-SCT between 2001 and 2011 from an HLA identical sibling donor (SIB) or a well-matched unrelated donor (MUD). Patients with transformed lymphoma, planned second transplants, allotransplants from cord blood, mismatched donors, and transplants with *ex vivo* T cell depletion (TCD) were excluded from the analysis.

**Results:** 1567 patients met the eligibility criteria (EBMT,  $n=1115$ ; CIBMTR,  $n=452$ ). The CIBMTR cohort had a higher proportion of MUD recipients [167 (37%) vs 252 (23%),  $p<0.001$ ], more cases with chemoresistant disease [113 (25%) vs 145 (13%),  $p<0.001$ ], less patients having received a prior auto-SCT [53 (12%) vs 403 (36%),  $p<0.001$ ], more use of myeloablative conditioning (MAC) (145 (32%) vs 220 (20%),  $p<0.001$ ) and less use of alemtuzumab *in-vivo* TCD [29 (6%) vs 201 (18%),  $p<0.001$ ] compared to the EBMT cohort. Median (range) follow up of survivors in months was 58 (3 – 130) and 54 (3 – 160) for CIBMTR and EBMT patients, respectively. All major outcomes [non-relapse mortality (NRM), relapse/progression (R/P), overall survival (OS) and progression free survival (PFS)] were comparable between the CIBMTR and EBMT samples. Multivariate analysis indicated that NRM was significantly affected by age (HR 1.04, 1.02-1.05,  $p<0.0001$ ), chemoresistant disease (HR 1.61, 1.28-2.03,  $p<0.0001$ ),  $\geq 5$  lines of prior CT (vs 3-4) (HR 1.62, 1.20-2.19,  $P=0.0015$ ) and Karnofsky performance score (KPS)  $< 80$  (HR 2.05 (1.32-3.19,  $P=0.0014$ ); R/P was significantly affected by grade 3 histology (HR 1.63, 1.16-2.26,  $P=0.0049$ ) and chemoresistant disease (HR 1.46 (1.07-1.97),  $P=0.0156$ ); PFS by grade 3 histology (HR 1.42, 1.15-1.76,  $P=0.0012$ ), chemoresistant disease (HR 1.54, 1.28-1.86,  $P<0.0001$ ),  $\geq 5$  lines of prior CT (vs 3-4) (HR 1.45, 1.13-1.85,  $P=0.0031$ ), MAC (HR 1.36,

1.14-1.63,  $P=0.0008$ ) and KPS  $< 80$  (HR 1.78, 1.23-2.58,  $P=0.0022$ ) and OS by age (HR 1.03, 1.02-1.04,  $P<0.0001$ ), grade 3 histology (HR 1.44, 1.13-1.83,  $P=0.0031$ ), chemoresistant disease (HR 1.59, 1.30-1.95,  $P<0.0001$ ),  $\geq 5$  lines of prior CT (vs 3-4) (HR 1.63, 1.25-2.13,  $P=0.0003$ ), MAC (HR 1.42, 1.16-1.73,  $P=0.0006$ ) and KPS  $< 80$  (HR 2.23, 1.52-3.25,  $P<0.0001$ ). Of note, outcomes between SIB and MUD were similar (3y OS 68% and 62%,  $P=0.114$ ).

**Conclusion:** This study represents an example of a fruitful cooperation between two important scientific transplant societies, the EBMT and CIBMTR. Despite significant differences in patient characteristics and transplant strategies between these 2 hitherto largest samples on allo-SCT for R/R FL, long-term disease control was similar and remarkably good with an R/P risk of about only 20% at 5 years. Chemoresistant disease, higher age, multiple pretreatment lines, poor KPS, and MAC all were predictors for an adverse outcome.

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#### O117

### Graft-versus-leukemia effects in T-prolymphocytic leukemia: evidence from MRD kinetics and TCR repertoire analyses

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**Introduction:** Preliminary clinical data suggest that allogeneic stem cell transplantation (alloSCT) may provide long-term disease control in a proportion of T-prolymphocytic leukemia (T-PLL) patients. However, direct evidence of graft-versus-leukemia (GVL) activity in T-PLL is lacking. We sought to investigate GVL by correlating minimal residual disease (MRD) kinetics with immune modulatory interventions (immunosuppression tapering, donor lymphocyte infusions (DLI), chronic graft-versus-host disease (cGVHD)), and T cell receptor (TCR) repertoire diversity alterations after alloSCT.

**Material (or patients) and methods:** The study sample consisted of 10 patients who received alloSCT for T-PLL at the University of Heidelberg between 2007 and 2015. Quantitative MRD monitoring was performed using clone-specific real-time quantitative PCR (RQ-PCR) of clonal TCR beta (TRB) and/or gamma gene rearrangements. TCR repertoire diversity was analyzed longitudinally by next-generation sequencing (NGS) on Illumina's MiSeq platform.

**Results:** All patients had a cytological complete response (CR) after alloSCT (5 unrelated, 4 related, 1 haploidentical). Conditioning was fludarabine with cyclophosphamide and/or total body irradiation-based. 2 patients died early because of acute GVHD, and one had no MRD marker, leaving 7 patients for MRD monitoring. Of these, 3 were MRD- at alloSCT, whereas 5 patients remained or became MRD+ early after alloSCT. In all of these 5 patients, immunosuppression tapering (3) or DLI (2) resulted in significant reduction of MRD levels (range 1-3 log) and was accompanied by cGVHD in 3 patients. However, durable MRD- was obtained in only 2 patients (alive 86+ and 12+ months post transplant), whilst MRD re-increased in 3 patients after 5-28 months despite ongoing cGVHD in one of them. The TRB repertoire of the three patients with the

longest follow-up (up to six years) was interrogated longitudinally before and after alloSCT using NGS. In all three patients, MRD decline was reproducibly associated with a shift from a clonal, T-PLL-driven profile to a polyclonal signature which largely corresponded to the donor TCR repertoire and receded with increasing MRD levels. Notably, there was no obvious correlation of GVL-induced MRD decline with emergence of particular dominant T cell clonotypes that could explain a clonal GVL effect. The 3-year relapse-free and overall survival of all 10 patients was 48% (95%CI 16-80%) and 58% (95%CI 27-90%), respectively.

**Conclusion:** This study provides first direct evidence for GVL activity in T-PLL, even though it appears to be often only limited or transient. Moreover, GVL in T-PLL does not seem to be driven by the emergence of novel dominant T cell clones but is rather relying on poly- or oligoclonal T cell responses. Nonetheless, alloSCT in T-PLL is a valuable treatment option, and further evaluation of MRD monitoring is warranted to optimize patient care after alloSCT.

**Disclosure of Interest:** None declared.

#### O118

##### **Reduced Intensity Conditioning transplantation (RIC) as part of first line therapy for Mantle cell lymphoma: results from the Phase II Mini Allo trial (CRUK: C7627/A9080)**

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**Introduction:** The current standard therapy for young patients with Mantle cell lymphoma involves high dose cytarabine based therapy followed by autologous stem cell transplantation (ASCT). This approach produces some impressive results with median overall survival >10 years in some series. However there is no plateau observed and at relapse, for suitable patients, an allogeneic transplantation can lead to long term disease free survival. We performed a trial evaluating the potential role of Reduced Intensity Conditioning transplantation (RIC SCT) as part of first line therapy for Mantle cell lymphoma (MCL). Patients were eligible for RIC SCT (conditioned with BCNU, etoposide, Ara-C, melphalan and alemtuzumab: BEAM-C) in first response, using either sibling or unrelated donor grafts. Donor lymphocytes were administered according to a standard protocol for persistent mixed chimerism or disease relapse. The primary endpoint of the study was progression free survival (PFS); a Fleming's single stage design was used with alpha of 0.1 and 90% power. We aimed to show that 2 year PFS could be increased from 45 to 70%, i.e. we would have 15/25 patients alive and progression free at 2 years.

**Material (or patients) and methods:** Twenty-five patients were recruited from 8 UK centres from January 2010 to September 2013. The median age was 54 years (range: 34-70), 22 (88%) were male. First line chemotherapy regimens were varied with 11(44%) patients achieving a CR and 14 (56%) a PR at the time of registration. Eleven (44%) donors were siblings and 15 (56%) were MUDs.

**Results:** All 25 received a RIC SCT and all had platelet and neutrophil recovery by day 100. Toxicity was as expected, with 96% reporting a grade 3-4 event, though only 2 patients died

from treatment related toxicity (TRM; 1 multi-organ failure and 1 GvHD). GvHD incidence was low in keeping with the T cell depletion. Only 3 patients developed Grade II acute GvHD (12%), and none developed Grade 3-4 GvHD; six (24%) developed extensive chronic GVHD. With a median follow-up of 35 months there have been 9 PFS events including 5 deaths (2 MCL, 2 TRM and 1 road accident). Fifteen patients were alive and progression free at 2 years with a further 2 patients progression free but yet to reach this point. The 2 year PFS estimate is 68% (95%CI: 46.1% - 82.5%). Of the patients who are alive and in remission, 14/16 have reached full donor T-cell Chimerism, 5 post DLI.

**Conclusion:** Our data demonstrate the feasibility of RIC SCT as consolidation of first response in MCL. We reached the target endpoint for PFS and believe that further exploration of RIC SCT in patients identified to be at high risk of disease progression with ASCT is warranted.

**Disclosure of Interest:** None declared.

#### O119

##### **The impact of advanced patient age on mortality after allogeneic hematopoietic stem cell Transplantation (alloHSCT) for Non-Hodgkin's Lymphoma (NHL): A retrospective study by the EBMT Lymphoma Working Party**

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**Introduction:** More than 60% of patients with NHL are over 60 years old at presentation and age remains a significant prognostic factor for outcome. An increasing number of NHL patients with pre-existing comorbidities are considered for alloHSCT. Data on the outcome of alloHSCT in elderly patients with NHL is sparse. The purpose of this study was to compare the potential risks and benefits of allogeneic HSCT in elderly patients with NHL with younger patients in a large sample taking also into account comorbidity information.

**Material (or patients) and methods:** Eligible for this EBMT LWP retrospective analysis, were all patients >= 18 years who had received an alloHSCT from a matched sibling or unrelated donor (cord blood and haplo excluded) for FL, DLBCL, MCL, or PTCL between 2003 and 2013 and were registered with the EBMT. **PRIMARY ENDPOINT** was 1-year non-relapse mortality (NRM); secondary endpoints were relapse incidence (REL); and overall survival (OS). **STATISTICAL ANALYSIS** was based on univariable and multivariable comparisons using stratified Cox and Fine & Gray regression models. OS was computed unadjusted by Kaplan-Meier and by multivariable adjustment using inverse probability weights (IPW).

**Results:** 3,919 patients were eligible and categorized by age: young (Y), 18-50y (n=1,772); old (O) 51-65y (n=1,967); very old (VO) 66-77y (n=180). Of the total 3,919 patients, 37% had FL, 30% DLBCL, 21 % MCL, and 11% PTCL; 85% were

chemosensitive and 15% chemorefractory at the time of alloHSCT, respectively. Comorbidity information (HCT-CI) was available for 979 patients. VO patients had a significant overrepresentation of diagnosis MCL, unrelated donor, prior treatment lines, prior autoHSCT and RIC conditioning. With a median follow-up for survivors of 3 years, NRM for Y/O/VO was 16%/20%/33% at 1 year and 18%/23%/37% at 2 years ( $P > 0.0001$ ), 2y-REL 27%/26%/23% ( $P = 0.429$ ), and unadjusted 2y-OS 61%/57%/43%. Multivariable adjustment for confounders including gender, NHL subset, time from diagnosis, chemosensitivity, donor, and conditioning confirmed that higher age was a significant predictor for NRM but not for REL, translating into a 2-year IPW-adjusted OS of 63%/56%/48% for Y/O/VO patients. Other independent significant predictors of NRM were chemorefractory disease, unrelated donor, and myeloablative conditioning; independent predictors of relapse were male gender, diagnosis DLBCL, chemorefractory disease, and less than 2y interval between diagnosis and alloHSCT. Although comorbidity and HCT-CI score were significant predictors of NRM in a subset analysis restricted to the 979 patients with comorbidity information available, age retained its significant impact on NRM but not on REL.

**Conclusion:** AlloHSCT in patients >65y exerts similar NHL control as in younger patients but is associated with a higher NRM which is not fully explained by comorbidity. Thus, although alloHSCT is feasible and effective in very old patients, the increased NRM risk has to be taken into account when deciding about transplant indication for NHL in this age group.

**Disclosure of Interest:** None declared.

#### O120

##### **Brentuximab-Vedotin (BV) as salvage treatment in Hodgkin Lymphoma patients (pts) before Autologous stem cell transplantation (ASCT) or failing ASCT: report based on 63 pts treated in Rete Ematologica Pugliese (REP-Italy)**

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**Introduction:** Historically High Dose Chemotherapy (HDC) and ASCT is the first option to treat recurrent HL pts with the possibility to achieve long term OS and DFS, ranging from 40 to 60% in chemosensitive pts. Nevertheless ASCT is limited to younger pts and results are not yet satisfactory in refractory or PET + pts after any salvage. In last 5 years BV a new antiCD30 MoAb is a real new challenge to rescue non responding pts.

**Material (or patients) and methods:** We report a retrospective analysis on 63 PET+ pts with HL treated with BV in Rete Ematologica Pugliese (REP-Italy). In 26 pts (41%) BV was used before ASCT and in 28 pts (45%) who failed long-term remission after ASCT. 9 pts (14%) received BV as consolidation after Ric Allo procedures. 53 pts (84%) received > 2 lines of CHT before BV. 40 pts (63%) were in stage  $\geq$  IIB prior to BV. 29 pts (46%) were considered refractory after  $\geq$  2 lines of CHT and 34 (%) relapsed (9.5 months median time to relapse) pre or post ASCT and before BV therapy (Tab. 1). All 63 pts were PET+ before BV.

**Results:** Median number of BV cures was 4 (3-8 range). Responses observed in whole group was CR, plus CRu in 27 pts (43%), PR in 15 pts (24%) with an ORR of 67%. No response or stable disease was demonstrated in 21% (33%). Responses were evaluated after 4 cures of BV. Regarding response according to timing of BV, CR were 13/26 (50%) in transplant naïve HL pts and 14/28 (50%) in pts relapsed after ASCT. In this last group 12 pts proceeded to Ric Allo transplant. Relapses after Ric allo were 5/12 and only 1 TRM was observed in the Allo transplanted cohort.

Table 1. HL pts characteristics and response

	N = 63	Percentage (%)
<b>Median age (range)</b>	31 (14-76)	
<b>Stage <math>\geq</math> IIB</b>	40	63
<b>PET+ at entry</b>	63	100
<b>&gt; 2 previous CHT before BV</b>	53	84
<b>BV timing pre ASCT</b>	26	41
<b>BV timing post ASCT</b>	28	45
<b>Consolidation post Allo</b>	9	14
<b>Response CR + Cru</b>	27	43 (ORR 67)
<b>Response PR</b>	15	24 (ORR 67)

**Conclusion:** Our data in a multicentric report support that BV can induce an impressive results (ORR67%) in HL relapsing/refractory pts. Probably better than classical salvage treatment as ifosfamide or Ara-C containing regimen. PET - was achieved in 43% of pts and no difference of response was observed in transplant naïve or in post ASCT group. BV is considered a really promising option to optimized sensitivity and reduce tumor burden in pts before ASCT and to induce an useful bridge to Ric/Allo in poor risk of HL pts.

**Disclosure of Interest:** None declared.

#### O121

##### **Gemcitabine, Fludarabine, and Melphalan for Reduced-Intensity Conditioning (RIC) and Allogeneic Stem Cell Transplantation (allo-SCT) for Relapsed/Refractory Hodgkin Lymphoma in the Brentuximab Vedotin Era**

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**Introduction:** In 2007 we elected to add gemcitabine (G), a highly active agent in Hodgkin Lymphoma (HL), to our standard fludarabine (F) - melphalan (M) RIC (1) in patients with relapsed/refractory HL undergoing allo-SCT to improve cytoreduction and hopefully reduce progressive disease (PD) after allo-SCT. Main G-associated non-hematologic toxicities include pulmonary, skin toxicities and nausea. Subsequently brentuximab vedotin (BV) was introduced as salvage therapy prior to allo-SCT to optimize responses pretransplant (2).

**Material (or patients) and methods:** Between 8/07 and 04/15, forty HL patients underwent an allo-SCT with the G-FM regimen. They had failed multiple conventional treatments (median prior chemotherapy regimens: 4), and a prior auto-SCT (77%). The median age was 31 years (range 20-63). Disease status at SCT was complete remission (CR) or undetermined (CRu) ( $n = 23$ ; 57%), partial remission (PR;  $n = 14$ ; 35%), and other ( $n = 3$ ; 7%). The median time to PD after auto-SCT was 6 months (range 1-68). Twenty-six patients (65%) received BV prior to allo-SCT, and in sixteen of them (40%) was the last therapy prior to allo-SCT. The overall response rate (CR/CRu) prior to allo-SCT was 65% (17/26) in BV-treated patients vs. 42% (6/14) in BV-naïve patients ( $P = 0.15$ ). The donor was a matched related donor (MRD;  $n = 21$ ) or matched unrelated donor (MUD;  $n = 19$ ). The conditioning regimen was G (800 mg/m<sup>2</sup> sq IV x1), F (33 mg/m<sup>2</sup> sq IV daily x4) and M (70 mg/m<sup>2</sup> sq IV daily x2). Thymoglobulin (4 mg/kg IV) was added in MUD allografts.

**Results:** At the latest follow-up (October 2015), thirty-one patients are alive. The median follow-up is 41 months (range 5-87). Twenty-seven surviving patients (87%) have a minimum follow-up of 12 months. Cumulative incidence of transplant-related mortality (TRM) at day 100 and three years are 15% and 17%, respectively. Nine patients expired (22%). Causes of death included graft rejection ( $n = 1$ ), acute GVHD ( $n = 1$ ), viral/

fungal pneumonia ( $n=3$ ); respiratory failure ( $n=1$ ), PD  $n=2$ , unknown ( $n=1$ ). Of the non-relapse related deaths, six occurred before day 100. Pulmonary toxicity (NCI CTC v3) was seen in 13 patients (33%). Grade 4-5 pulmonary toxicity was seen in five (13%). Otherwise it was grade 1-3 ( $n=8$ ). Skin toxicity was seen in 11 patients (28%: grade 3-4  $n=2$ ; grade 1-2  $n=9$ ). Nausea was seen in 37 patients (92%). It was grade 1-2 in all of them. The overall response rate (CR/CRU) after allo-SCT was 81% (21/26) and 86% (12/14) in BV-treated and in BV-naïve patients ( $P=0.5$ ). At three years, overall and progression-free survival (OS/PFS) are 75% (95% CI: 57-86) and 54% (95% CI: 36-70), respectively. Cumulative overall PD incidence is 28% (95% CI 16-50).

**Conclusion:** G-FM140 allows moderate dose-intensification of the preparative regimen in these high-risk patients with acceptable morbidity and mortality. The inclusion of gemcitabine affected nausea, pulmonary and likely skin toxicity but TRM remained low. We can confirm that exposure to BV may allow more patients to reach allo-SCT in complete remission (2). With over 50% of patients progression-free at 3 years, RIC allo-SCT in HL remains an effective and relevant treatment option even in the BV era.

**References:** 1. Anderlini *et al*, Haematol 2008; 93:257

2. Chen *et al*, Blood 2012; 119:6379.

**Disclosure of Interest:** None declared.

## O122

### Haploidentical peripheral blood transplantation in advanced and active Hodgkin Lymphoma: results with Treosulfan-based conditioning

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**Introduction:** Advanced and refractory Hodgkin Lymphoma (HL) represents a medical need and allogeneic stem transplantation is considered among treatment options.

**Material (or patients) and methods:** Between March 2007 and September 2015, 30 patients (pts) with advanced HL received unmanipulated peripheral blood stem cell transplantation from related haploidentical donor (haploSCT) with myeloablative Treosulfan based conditioning regimen. Median age at haploSCT was 30 years, male 53% (16); Only 2 patients underwent haploSCT without previous Autologous SCT. At haploSCT, 22 patients (73%) had detectable disease, and 16 of those were in stable/progressive disease.

The backbone conditioning regimen consisted of Treosulfan (14 g/m<sup>2</sup>/day) on days -6 to -4 and Fludarabine (30 mg/m<sup>2</sup>/day) on days -6 to -2 and 15 pts were treated accordingly (TrRAMM).

TBI2 Gy/day on days -1 and 0 was added in 5 patients (TrAMM4Gy), Melphalan (70mg/mq/day) on days -2 and -1 in 9 patients (TFM) and Thiotepa 5mg/kg on days -4 and -3 in 1 patient (TTF). GvHD prophylaxis consisted of anti-Thymocyte globulin 10mg/Kg from day -4 to -2 plus Rapamicin from day -7 to +90 and MMF from day 0 to +30 for TrRAMM and TrRAMM4Gy regimen. In TFM and TTF regimens pts received Cy 50mg/Kg on days +3 and +4, Rapamicin from day +5 to +90 and MMF from day 0 to +30. The median numbers of infused CD34+/Kg were 6.38 x 10<sup>6</sup> (range 4.46-10.94) and median numbers of infused CD3+/Kg were 2.41 x 10<sup>8</sup> (range 1.25-4.76).

**Results:** Twenty-six patients were evaluable for engraftment; the median time to neutrophil  $\geq 0.5 \times 10^9/L$  was 18 days (range 13-32) and 17 days (range 10-68) to platelet  $\geq 20 \times 10^9/L$ . No graft failure was observed. Chimerism was evaluable in 26 patient; on day +30 all patients had 100% donor chimerism on marrow cells. Median follow-up was 871 days. One- and 3-years OS was 71 and 47 %, PFS was 54%

and 29% at 1 and 3 years respectively; cumulative incidence of relapse was 40% and 60% at 1 and 3 years. In our cohort of patients overall survival and progression free survival was not statistically different when patients were stratified for disease phase, refined Disease Risk Index or HCT-CI. Non-relapse mortality (NRM) was 10% at 100 days and 14% at 180 days and at one year. Ten (33%) patients had aGvHD grade 2-4, while 9 (27%) patients moderate-severe cGvHD. CMV reactivation occurred in 9 patients; no CMV disease was observed.

Fifteen patients received chemotherapy after allo SCT for relapse of their disease; 8 of them received also one or more DLI (median 1, range 1-3) following chemotherapy. After post transplant treatment 7 patients were alive and 5 of them were disease free at last follow-up. At last follow-up, 16 patients are alive and 13 of them are in complete remission.

**Conclusion:** Unmanipulated haploidentical peripheral blood transplantation after treosulfan-based myeloablative conditioning is a valid treatment option in advanced, active Hodgkin disease and allows concomitant post haploSCT therapy with beneficial effects on overall survival.

**Disclosure of Interest:** None declared.

## O123

### Identification of baseline characteristics that predict good outcome of alloSCT in young CLL patients - a retrospective analysis from the CMWP of EBMT

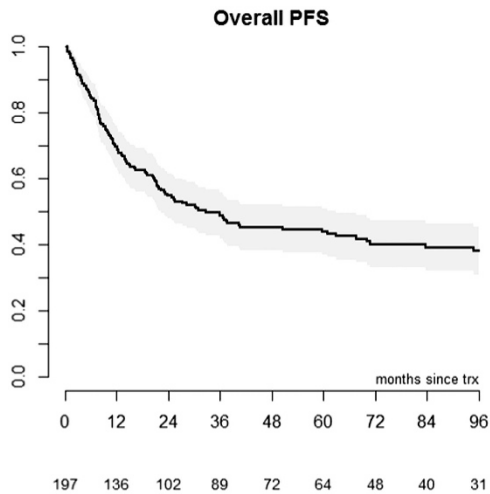
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**Introduction:** AlloSCT as treatment of high risk CLL patients is under debate. Results of treatment with BTK and PI3K inhibitors are excellent, although patients with del(17p) experience decreased EFS and OS. AlloSCT may compete with the targeted therapies especially when young. A precondition for better outcome after alloSCT is low NRM. This study was set up to identify relevant pre-transplant factors predicting low NRM and high EFS in young (< 50 years of age) transplanted CLL patients.

**Material (or patients) and methods:** We performed Cox regression analyses in order to identify risk factors for NRM and EFS in an updated EBMT registry cohort containing 197 CLL patients aged less than 50 years.

**Results:** Median age was 46 years (81%  $\geq 40$ ), 40% had purine-analogue refractory disease and 19% had relapsed after purine-analogue combination therapy. 17% had del(17p) and



35% del(11q). Responsive disease was present in 62%. Median follow-up for the whole cohort is 90 months. 2-, 5- and 8-year EFS were 54%, 43% and 38% (median 32.7 months). In multivariate analysis the following factors were significantly associated with inferior EFS: prior Autologous SCT (HR 2.0,  $P=0.02$ ), no remission at the time of alloSCT (HR 2.9,  $P < 0.01$ ) and mismatched unrelated donor (HR 3.9,  $P < 0.01$ ). In vivo TCD with ATG, mostly applied for unrelated donors, improved EFS (HR 0.55,  $P=0.04$ ). There was a trend for improved EFS when not having del(17p) or del(11q) [HR 0.58,  $P=0.09$ ]. Cumulative incidence of 2-year NRM was 23% (95%CI 17-29%). In multivariate analysis, predictors for NRM risk were unrelated donors and female donor for male patient.

**Conclusion:** Two year EFS for young transplanted CLL patients is similar as for ibrutinib treated patients with del(17p). Low NRM is predicted when no HLA-mismatched unrelated or sex mismatched donors for male patients are used. The current pressing question is if patients, especially those with del(17p), and with a low risk for alloSCT-related NRM will benefit most from alloSCT including the use of targeted therapy in the event of relapse, or from a non-alloSCT treatment strategy with targeted therapies (ibrutinib, idelalisib, venetoclax) when available, given consecutively in case of relapse. Further analyses are directed to show NRM and long-term EFS of subgroups of transplanted patients with del(17p) with "good transplant risk factors" like being in remission at the time of alloSCT and use of well-HLA-matched donors as the best possible attempt to guide clinical decision making.

**Disclosure of Interest:** None declared.

## O124

### Impact of procedure related factors and center effects on outcome of CLL transplantations

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**Introduction:** Even with novel targeted therapies for the treatment of Chronic Lymphocytic Leukemia (CLL) patients, such as BTK, PI3K and BCL2 inhibitors, allogeneic hematopoietic stem cell transplantations (HCT) will remain an important treatment option, and information on patient and procedure-related risk factors is valuable. In a previous study we investigated the impact of patient and disease related risk factors on outcomes after HCT. Taking these into account, we explored the impact of procedure and center related factors.

**Material (or patients) and methods:** Data were analyzed from 684 CLL patients who received a first HCT between 2000 and 2011 and who were part of the EBMT CLL Data Quality Initiative, where data on baseline risk factors was collected and outcome information was updated.

Event-Free Survival (EFS) up to 5 years after transplantation, mortality in the first 100 days after HCT and mortality after relapse after HCT were investigated. Outcomes were analysed using the Kaplan-Meier method and Cox proportional hazard models, with a frailty component to account for unexplained center heterogeneity. Risk factors considered included age at HCT, previous autologous transplantation, conditioning, cytogenetics, performance status, remission status, donor-patient sex match, donor HLA match, T-cell depletion (TCD) and transplantation year on the patient level, experience in HCT in general and in CLL in particular on the center level. Gross National Income (GNI) and Health Expenditure per capita (purchasing power parity) data were also considered. Missing baseline data were handled by multiple imputation models.

**Results:** Five-year EFS of the whole cohort was 37% (95% Confidence Interval 33%>42%), day 100 survival was 90% (88%>92%) and survival after relapse 32% (25%>41%) at 3 years after relapse (based on  $n=178$ ). Transplant experience was measured by the number of all HCTs, resp. of CLL HCTs, in the center in which the patient was transplanted in the year before his/her HCT. These numbers ranged from 2 to 166 (median 54.5), resp. 0 to 16 (median 3). Larger numbers of CLL transplants in the previous year (Hazard Ratio 0.97 per additional transplant,  $P=0.10$ ) and GNI per capita (HR 0.90 per additional \$10,000,  $P=0.10$ ) showed a protective impact on 5-year EFS in a model also considering other risk factors. In vivo TCD with alemtuzumab (HR 1.7 compared to no TCD,  $P=0.005$ ) and a female donor for a male patient (HR 1.3 compared to a male donor for a male patient,  $P=0.03$ ) were the only procedure-related factors significantly associated with EFS. Even when correcting for patient and center characteristics, there was still significant variation in center outcome left, expressed by center-specific model-derived hazard ratios ranging from 0.6 to 1.2. CLL experience (HR 0.96 per additional transplant,  $P=0.03$ ) had a significant impact on survival after relapse.

**Conclusion:** This study shows the large contribution of known and unmeasured center characteristics on different outcomes after transplantation, also when taking into account differences in patient mix. Since centers differ in their treatment strategies and selection of patients, the influence of procedure related factors and center characteristics can only partially be disentangled. These results may help improve the



interpretation outcomes of single or multicenter studies and may improve prediction of the prognosis of candidates for HCT.

**Disclosure of Interest:** None declared.

## O125

### Comparison of outcome after haplo-identical, HLA-mismatched unrelated donor and unrelated cord blood transplantation in patients with myelodysplastic syndrome (MDS) and secondary acute myeloblastic leukemia (sAML): an EBMT study

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**Introduction:** Higher risk MDS patients who have no HLA-matched sibling donor or an unrelated HLA-matched donor may be transplanted from an HLA-mismatched donor either an unrelated donor, a related donor or a cord blood and the "best donor" is unknown up to now. The aim of the current study is to compare outcome of patients who received either an HLA-mismatched related graft considered as haplo-identical, an HLA-mismatched unrelated donor or an unrelated cord blood thanks to EBMT registry.

**Material (or patients) and methods:** 1491 patients receiving a first transplant from mismatched related (RD,  $n=621$ ), mismatched unrelated (UD,  $n=428$ ) or unrelated cord blood (CB,  $n=442$ ) from January 2003 to June 2015 were included in the present study. Adjusted overall survival (OS), non-relapse mortality (NRM) and relapse incidence (RI) were compared in the 3 groups: RD, UD and CB.

**Results:** Median age of the whole population was 53 years at time of transplant (range 18 to 76). There were 1007 patients with a primary diagnostic of MDS and 484 with a secondary leukemia at time of diagnosis. 294 (29%) MDS patients were transformed into AML at time of transplantation. 627 (44%) patients were considered in complete remission at time of transplant (68 missing). Median time from diagnosis to transplant was 8 months. 737 (50%) patients received a reduced intensity conditioning regimen and 630 (43%) patients received total body irradiation. GVHD prophylaxis was cyclosporine-based for 953 (64%) patients. Post-transplant cyclophosphamide concerns 25% of mismatched related transplant. *In vivo* T depletion was used in 669 (53%) patients. RD, UD and CB patients differed in particular regarding gender, disease, blast count at transplant, status at transplant, RIC vs MAC, gender mismatch and *in-vivo* T-cell depletion.

Cytogenetic was available for 220 patients only. Median follow-up was 13 months for RD, 72 months for UD and 31 months for CB. Cumulative incidence of grade II-IV acute GVHD was 11%, 27% and 23% in RD, UD and CB. Engraftment was observed for 87%, 85% and 80% for RD, UD and CB. OS at 72 months was 27, 32 and 27% for RD, UD and CB. NRM was 46, 46 and 48% for RD, UD and CB. Relapse incidence was 27, 24 and 28% for RD, UD and CB. After adjustment for period, age, gender, disease type, time from diagnosis, status at transplant, gender mismatched, CMV, conditioning regimen (reduced versus myelo-ablative) and *in vivo* T depletion, there were significant differences for OS and NRM in favor of UR, the worse outcome being with CB use. With RD as a reference, hazard ratio (HR) for OS was 0.72 for UD and 1.17 for CB ( $P=0.0009$ ); HR for NRM was 0.76 for UD and 1.20 for CB ( $P=0.016$ ); HR was 0.74 for UD and 1.18 for CB ( $P=0.071$ ). RD patients receiving cyclophosphamide had better OS than those not receiving cyclophosphamide ( $P=0.0006$ ), with 72 mo. OS of 41% vs 26%. NRM was also lower, 21% vs 48% ( $P < 0.0001$ ), whereas the cumulative incidence of relapse did not differ markedly, 30% vs 26% ( $P=0.83$ ).

**Conclusion:** To conclude, in MDS and sAML patients without an HLA matched sibling donor, outcome was better after UD than RD or CB. Considering only patients who received a graft from a RD and post-transplant cyclophosphamide, results seem very encouraging with the best outcome with the greatest improvement in NRM.

**Disclosure of Interest:** None declared.

## O126

### Ruxolitinib-induced spleen size reduction predicts superior outcome after allogeneic stem cell transplantation in primary and post-PV/ET myelofibrosis – a study of the Cooperative German Transplant Study Group (KTS)

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**Introduction:** Ruxolitinib reduces spleen size and constitutional symptoms (CS) in patients with myelofibrosis, but its impact on subsequent allogeneic stem cell transplantation (allo-HCT) is not sufficiently defined. We examined the effect of ruxolitinib therapy prior to transplant on early outcome after allo-HCT in 52 patients with primary or secondary post-PV/ET myelofibrosis patients.

**Material (or patients) and methods:** This retrospective multicentre study was performed within the cooperative German transplant group (KTS). Clinical data were evaluated by a questionnaire, data were recorded and analyzed by SPSS.

**Results:** 52 patients with primary ( $n=39$ ) or post ET/PV myelofibrosis ( $n=13$ ) and a median age at transplant of 60 years (range 35–74) received ruxolitinib prior to allogeneic stem cell transplantation in 7 german centers. The median follow-up of patients after allo-HCT is 15.8 months. According to the DIPSS risk classification, patients were classified as intermediate-1 ( $n=4$ ), intermediate-2 ( $n=39$ ), or high risk ( $n=9$ ). 36 patients were JAK2 V617F, 5 Calreticulin Exon 9 and 3 JAK Exon 12 mutated. 15 patients had an aberrant karyotype. 14 patients stopped ruxolitinib before the start of conditioning, 5 patients tapered ruxolitinib prior to SCT and 7 suffered from symptom rebound. All other patients ( $n=33$ ) received ruxolitinib until the day before starting conditioning. Most patients received stem cells (PBSCT:  $n=47$ , BM:  $n=5$ ) from

matched-unrelated ( $n=31$ ) donors, 6 received grafts from matched-related, 13 from mismatch-unrelated and 1 from a haploidentical donor. 41 patients were conditioned with BuFlu, 7 with FluMel, 2 FLAMSA-based, 1 received TBF. Most patients received CyA/MMF as primary immunosuppression. The incidence of acute GvHD Grad 2-4 was 56.9%, extensive chronic GvHD was seen in 45.1%. Graft failure and poor graft function rates were 16% and 14%, respectively. Only 4 patients relapsed after allo-SCT and OS rate of the overall cohort was 70%. When focusing on OS according to response to ruxolitinib, spleen size reduction of >25% plus/minus response of CS is associated with a significantly improved OS after allo-SCT (spleen size + CS improvement: OS probability 93% vs. 67% at 12 months and 75% vs. 50% at 24 months,  $P=0.039$ ; spleen size response only w/o CS: OS probability 94% vs. 66% at 12 months and 75% vs. 48% at 24 months,  $P=0.026$ ). If spleen size responses are grouped in < 25% ( $n=31$ ), 25-50% ( $n=12$ ) and >50% ( $n=6$ ) reduction, a clear spleen size reduction-related effect on OS can be seen (OS probability at 12 months 100% vs. 92% vs. 77% and at 24 months 100% vs. 69% vs. 48%), which however (due to the limited number of patients in the top-responder group) did not reach statistical significance ( $P=0.07$ ). Response of CS without including spleen size reduction does not sufficiently split the OS curve (OS probability 82% vs. 67% and 68% vs. 44% at 12 and 24 months respectively,  $P=0.26$ ).

**Conclusion:** Ruxolitinib-induced spleen size reduction of >25% at time of transplant predicts outcome after allo-SCT in primary and post-ET/PV MF. This data suggests indication of allo-SCT at the time of best spleen response during ruxolitinib therapy in patients with an indication for allo-SCT (i.e. DIPSS int-2 and high risk patients having a suitable donor).

**Disclosure of Interest:** D. Wolf Conflict with: Novartis (speaker honorary), J. Jonas: None declared, A. Haifaa: None declared, G. Wulf: None declared, E. Wagner: None declared, M. Bornhäuser: None declared, T. Schroeder: None declared, M. Crysant: None declared, K. Mayer: None declared, P. Brossart: None declared, M. Christopheit: None declared, F. Ayuk: None declared, N. Kröger Conflict with: Novartis (speaker honorary).

## O127

### Impact of molecular genetics on outcome in patients with myelofibrosis following allogeneic stem cell transplantation

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**Introduction:** Primary or post-ET/post-PV myelofibrosis is one of the Philadelphia chromosome-negative chronic myeloproliferative neoplasia characterized by significantly reduced overall survival.

Allogeneic stem cell transplantation is still the only curative treatment option for patients with myelofibrosis but the impact of molecular mutation on outcome has not been studied so far.

**Material (or patients) and methods:** Samples from 169 patients with a median age of 58 years (r: 18-75) who received allogeneic SCT either from related ( $n=36$ ) or unrelated ( $n=133$ ) donor were analyzed. The intensity of conditioning was mainly reduced intensity ( $n=166$ ), rather than myeloablative conditioning ( $n=3$ ). Patients suffered from primary myelofibrosis ( $n=110$ ), post-ET/PV myelofibrosis ( $n=46$ ), while 13 patients were in acceleration or had transformed into acute

myeloid leukemia. According to dynamic IPSS (DIPSS) ( $n=165$ ) the patients were either low ( $n=7$ ), intermediate-1 ( $n=35$ ), intermediate-2 ( $n=91$ ), or high risk ( $n=32$ ). Regarding molecular genetics we found JAK2V617F mutations in 62%, calreticulin (CALR) mutations in 20%, MPL mutations in 4%, U2AF1 in 7%, SRSF2 in 10%, SF3B1 in 4%, ASXL1 in 29%, IDH1 in 2%, IDH2 in 3%, CBL in 1%, DNMT3A in 4%, TET2 in 10%, EZH2 in 4%, while none of the patients showed mutations in ETV6 and PTPN11. Overall, only in 11 patients no mutation could be detected. One mutation could be detected in 41%, 2 mutations in 30%, 3 mutations in 11%, 4 mutations in 5%.

**Results:** During follow-up 39 patients experienced relapse and 46 patients experienced non-relapse mortality.

From the non-molecular factors regarding progression-free survival (PFS) in univariate analysis, age < 58 ( $p < 0.01$ ), intermediate-1 and low risk according to DIPSS ( $P=0.002$ ), HLA-matched vs. mismatched ( $P=0.04$ ) were significant factors for improved PFS. Regarding molecular markers improved disease-free survival was seen for patients with mutations in CALR ( $P=0.005$ ), while negative impact on PFS was seen for mutations in U2AF1 ( $P=0.035$ ), ASXL1 ( $P=0.05$ ), IDH2 ( $P=0.006$ ), DNMT3A ( $P=0.029$ ). No significant difference could be seen for patients with EZH2, IDH1, SRSF2, and SF3B1 mutations. There was no difference in PFS for patients without any mutation vs. 1, and more than 1 mutation ( $P=0.12$ ). Regarding the previously described unfavorable mutations ASXL1, SRSF2, EZH2, IDH1, and IDH2, we found 40 patients who had at least 1 of these unfavorable mutations, 11 had 2 of these mutation, and 1 had 3 of these unfavorable mutations. However, the estimated 5-year PFS did not differ significantly between patients without any of these unfavorable mutations, with 1 or with 2 of them (47 vs. 40 vs. 41%,  $P=0.5$ ).

In a multivariate analysis for PFS survival beside higher age (HR 2.134), DIPSS intermediate 2 and high risk (HR 1.711), HLA mismatch (HR 1.474), IDH2 (HR 5.451), and ASXL1 (HR 1.532) influenced PFS negatively, while CALR mutation resulted in a significantly improved PFS (HR 0.393,  $P=0.01$ ).

**Conclusion:** The poor prognosis of the recently described unfavorable mutated genes SRSF2, EZH2, and IDH1 was not observed and may therefore be overcome by allogeneic SCT. However, in a multivariate analysis for PFS only IDH2 and ASXL1 reduced PFS while CALR mutation resulted in improved PFS.

**Disclosure of Interest:** None declared.

## O128

### Outcome of Patients with Myelofibrosis Relapsing after Allogeneic Stem Cell Transplant: A Retrospective Study by the Chronic Malignancies Working Party of EBMT

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**Introduction:** Over the last decade, there has been a significant increase in the number of patients with Myelofibrosis (MF) undergoing allogeneic stem cell transplantation (SCT). However, scarce information exists on the outcome of patients who relapse following SCT. We conducted a retrospective EBMT registry analysis of MF patients who relapsed following first SCT.

**Material (or patients) and methods:** A total of 1065 adult patients (849 (80%) with Primary MF (PMF) and 216 (20%) with secondary MF (sMF)) underwent 1<sup>st</sup> allogeneic SCT between 2000 and 2010. A total of 251 (24%) patients from this cohort (206 PMF and 45 sMF) had documented relapse  $\geq$  day 30 after HSCT and were included in the analysis. There were 163 males and 88 females; median age was 55 years old (range 21.5-70). A total of 84 patients (33%) had received Myeloablative Conditioning (MAC) and 167 patients (67%) Reduced Intensity Conditioning (RIC). There were 123 matched siblings (49%) and 128 unrelated donors (51%). Acute GVHD (aGVHD) status was available for 97% of patients; no aGVHD in 142 patients, Grade I-II 76 patients, Grade III-IV 22 patients and 2 patients with aGVHD ungraded.

**Results:** Median time to relapse after SCT was 7.1 months (range 1-111). Median Overall Survival (OS) from time of relapse was 17.7 months (95% Confidence Intervals 11-24). Collectively, there was a significant difference in survival outcome for those relapsing  $>$  7.1 months post-SCT (median survival 30.3 months post relapse) compared to those relapsing within 7.1 months following the SCT episode (median survival 7.9 months post relapse;  $P < 0.001$ ). Absence of aGVHD or grade I aGVHD only was associated with a trend towards improved survival following relapse compared to those with Grade II-IV aGVHD ( $P = 0.12$ ). For PMF, disease duration prior to SCT did not significantly affect outcome post relapse. Heterogeneous practice existed as regards management of the relapse episode, with considerable variation in median survival (MS) estimates. 47 patients received Donor Lymphocyte Infusions (DLI) alone (MS 76 months); 21 had chemotherapy alone (MS 23 months) whereas 14 patients had DLI combined with chemotherapy (MS 13.6 months). As regards 2<sup>nd</sup> allografts: 53 patients underwent 2<sup>nd</sup> allograft alone (MS 23.6 months) and 26 underwent DLI and 2<sup>nd</sup> SCT (MS 53.9). In 90 patients active management – if any – was not documented (most likely many were palliative) but represented a very poor risk group with a MS of only 4.8 months. Overall, there was a significant improvement in OS post relapse for those undergoing 2<sup>nd</sup> SCT ( $n = 79$ ) versus those who did not have a 2<sup>nd</sup> SCT ( $n = 172$ ;  $P = 0.019$ ).

**Conclusion:**

Treatment of relapse presents huge challenges and the heterogeneous management strategies highlighted above reflects current practice where approaches range from palliation through to intensive chemotherapy and 2<sup>nd</sup> SCT. It is clear from this analysis that early relapse has a much worse prognosis than those who relapse later than 7.1 months post-SCT. There is a definite survival advantage for those who undergo DLI and/or a 2<sup>nd</sup> SCT procedure, although we acknowledge that those patients undergoing a 2<sup>nd</sup> SCT represent a highly selected group who are fit enough to undergo such intervention. Moreover, how relapse management practice will change in the era of novel therapies such as JAK inhibitors to bridge towards 2<sup>nd</sup> SCT is currently unclear.

**Disclosure of Interest:** None declared.

**O129**

**Hematopoietic cell transplantation in Myelodysplastic Syndrome after Failure of Hypomethylating Agents**

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**Introduction:** The outcome of patients with myelodysplastic syndromes (MDS) after failure of therapy with

hypomethylating agents (HMA) is poor<sup>1</sup>. Allogeneic hematopoietic cell transplantation (HCT) seems to be effective in this setting, but its role is not well defined.

**Material (or patients) and methods:** Among 289 patients transplanted between June 2004 (FDA approval of Azacitidine) and December 2013 at Fred Hutchinson Cancer Research Center (FHCRC) we identified 65 patients who were transplanted after HMA failure. Failure was defined as loss of response, progression to higher risk MDS or acute myeloid leukemia (AML), or no hematological improvement after at least 4 HMA cycles. To define response, we used the 2006 modified International Working Group (IWG) response criteria<sup>2</sup>. Among the remaining 224 MDS patients, we identified 60 HMA responders. Response was defined as at least hematological improvement after HMA treatment. Of note, 55 of 60 patients were in complete remission (CR) or Marrow CR at HCT. We compared outcomes of the two groups using Cox regression. We used a forward stepwise method to select the variables for our models. Candidate variables included gender, age at HCT, secondary MDS, revised International Prognostic Scoring System (IPSS-R) at diagnosis, pre-HCT evolution to AML, conditioning intensity (low versus high), and stem cell source.

**Results:** Overall, 73 of the 125 patients (58%) had died by the time of last contact. Median follow-up of survivors measured from HCT was 41.9 months (range, 2.7 – 98.5). The unadjusted 3-year estimates of overall survival (OS) were 34.1% versus 47.9% among HMA failures versus responders, respectively ( $P = 0.19$ , Log rank test). After adjustment of covariates, HMA failures showed similar OS compared to responders [Hazard ratio (HR) 1.55, 95%CI, 0.89 – 2.69,  $P = 0.19$ ]. The unadjusted 3-year estimate of relapse-free survival (RFS) was significantly lower for HMA failures than for responders, 23.3% versus 41.4%, respectively ( $P = 0.03$ , Log rank test). After adjustment for covariates, the Cox regression model showed a trend to lower RFS of HMA failures compared to responders [HR 1.65, 95%CI, 0.98 – 2.79,  $P = 0.06$ ]. The 3-year cumulative incidence of transplant-related mortality (TRM) was 22.3% and 22.6% among HMA failures and responders, respectively (HR 1.21, 95%CI, 0.57 – 2.59,  $P = 0.62$ ). Conversely, the 3-year cumulative incidence of relapse was significantly higher in patients HMA failures than among responders, 56.1% versus 36%, respectively, HR 2.24, 95%CI, 1.27 – 3.97,  $P < 0.01$ .

**Conclusion:** HCT is a feasible option for patients who failed HMA therapy. No increased TRM in HMA failures was observed compared to responders. Patients who have failed HMA have a higher risk of relapse compared to patients who have responded to HMA. Clinical Trials evaluating novel conditioning regimens and maintenance strategies should be considered in patients who have failed HMA.

**References:** 1. Prebet et al, JCO, vol. 29, issue 24, 322-27.

2. Cheson et al, Blood, 2006, vol. 108, 419-25.

**Disclosure of Interest:** None declared.

**O130**

**Early Initiation of Defibrotide (DF) Improves Survival in Hepatic veno-occlusive Disease/sinusoidal Obstruction Syndrome (VOD/SOS) with or without multi-organ dysfunction (MOD) Post Hematopoietic Stem Cell Transplantation (HSCT): Updated Interim Results**

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**Introduction:** VOD/SOS is an unpredictable, potentially life-threatening complication of conditioning for HSCT.

[0130]

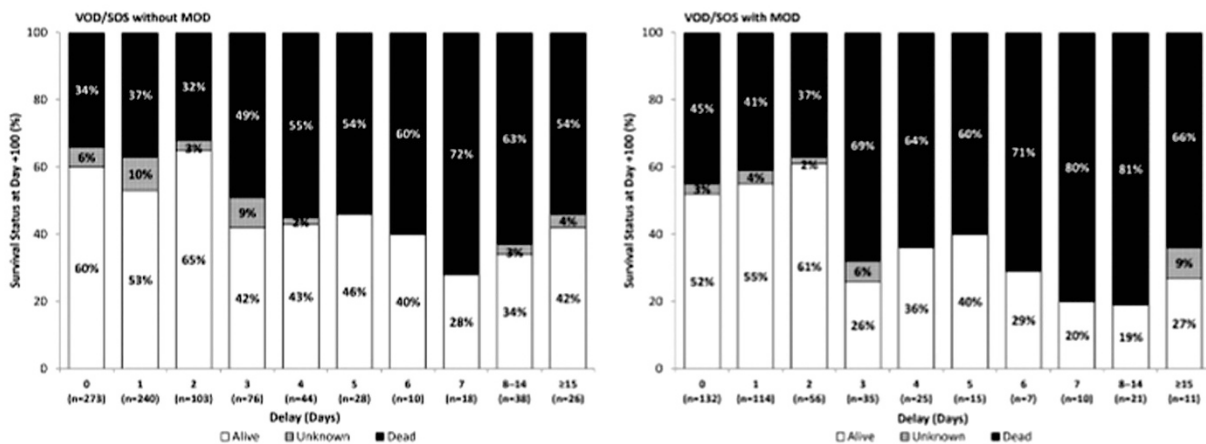
Table. Day+100 Survival by Cut-Point

Initiation Day	HSCT VOD/SOS (N = 755)				HSCT VOD/SOS with MOD (n = 426)			
	Alive	Died	Unknown	Difference (95% CI) P value <sup>a</sup>	Alive	Died	Unknown	Difference (95% CI) P value <sup>a</sup>
≤ 1	249 (54.8)	172 (37.9)	33 (7.3)	11.9% (4.4-19.3%)	132 (53.7)	106 (43.1)	8 (3.3)	16.3% (6.5-25.7%)
> 1	137 (45.5)	153 (50.8)	11 (3.7)	.002	69 (38.3)	107 (59.4)	4 (2.2)	.001
≤ 2	310 (56.5)	203 (37.0)	36 (6.6)	22.0% (13.9-30.0%)	166 (55.0)	127 (42.1)	9 (3.0)	27.7% (17.3-37.8%)
> 2	76 (36.9)	122 (59.2)	8 (3.9)	< .001	35 (28.2)	86 (69.4)	3 (2.4)	< .001
≤ 3	334 (54.6)	237 (38.7)	41 (6.7)	21.4% (12.2-30.4%)	175 (51.9)	151 (44.8)	11 (3.3)	24.1% (12.5-35.5%)
> 3	52 (36.4)	88 (61.5)	3 (2.1)	< .001	26 (29.2)	62 (69.7)	1 (1.1)	< .001
≤ 4	351 (53.8)	260 (39.8)	42 (6.4)	22.4% (11.9-32.8%)	184 (50.8)	167 (46.1)	11 (3.0)	25.4% (12.1-38.4%)
> 4	35 (34.3)	65 (63.7)	2 (2.0)	< .001	17 (26.6)	46 (71.9)	1 (1.6)	< .001
≤ 7	369 (52.6)	291 (41.5)	42 (6.0)	22.6% (8.3-36.7%)	194 (49.2)	189 (48.0)	11 (2.8)	28.1% (9.8-46.1%)
> 7	17 (32.1)	34 (64.2)	2 (3.8)	.002	7 (21.9)	24 (75.0)	1 (3.1)	.003
≤ 14	378 (51.5)	313 (42.6)	43 (5.9)	14.7% (-7.7-37.2%)	198 (47.7)	206 (49.6)	11 (2.7)	19.0% (-13.4-53.4%)
> 14	8 (38.1)	12 (57.1)	1 (4.8)	.255	3 (27.3)	7 (63.6)	1 (9.1)	.340

<sup>a</sup>Fisher's exact test, alive and died.

[0130]

Figure. Day +100 Survival by Day of Dosing



Severe VOD/SOS (ie, with MOD) may be associated with > 80% mortality. Defibratide (DF) is approved for treatment of severe VOD/SOS in the EU. In the US, a new drug application for DF proposed for the treatment of hepatic VOD/SOS with MOD was filed in 2015. Currently in the US, DF is available only through an ongoing, expanded-access study. The impact of time to DF treatment initiation (TI) is of interest.

**Material (or patients) and methods:** In the expanded-access study, patients (pts) with VOD/SOS (Baltimore/modified Seattle criteria or biopsy) with/without renal/pulmonary MOD received DF 25 mg/kg/d in 4 divided doses for ≥ 21 days. Day+100 survival in HSCT pts was examined *post hoc* based on time from VOD/SOS diagnosis to DF TI. Two analyses were conducted: (1) survival rate analyzed by TI for all pts before or after days 1, 2, 3, 4, 7, and 14, using Fisher's exact test; (2) survival rate for only those pts with TI on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8-14, and ≥ 15 (Cochran-Armitage test for trend across days).

**Results:** Among HSCT pts enrolled through April 18, 2015, who received ≥ 1 DF dose, TI date was available for 755 pts including 426 with MOD. DF was started on the day of diagnosis in 31.7% of pts; 93.0% of pts started DF on or before day 7 post-diagnosis.

In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14, earlier DF TI was associated with higher survival rates (Table), and was statistically significant for all cut-points except day 14, with only 2.8% of pts with TI post-day 14. In the analysis of relationship between Day+100 survival and TI day, there was a statistically significant trend over time for higher Day+100 survival with earlier initiation ( $P < .001$ ; Fig).

**Conclusion:** Data indicate decreased Day+100 survival associated with longer treatment delays, confirmed by the Cochran-Armitage test ( $P < .001$ ). Thus, DF should be initiated as soon as possible after VOD/SOS diagnosis, as no day post-diagnosis provides a viable cut-point for better outcome.

**Support:** Jazz Pharmaceuticals.

**Disclosure of Interest:** P. Richardson Funding from: Received grants from Gentium SpA during the conduct of the study, Conflict with: Served on advisory committees for Gentium SpA/Jazz Pharmaceuticals, A. Smith: None declared, B. Triplett: None declared, N. Kernan: None declared, S. Grupp Personal Interest: Served as a consultant to Jazz Pharmaceuticals, J. Antin Personal Interest: Served on advisory committees with Jazz Pharmaceuticals, L. Lehmann: None declared, M. Miloslavsky Employee of: Jazz Pharmaceuticals; in the course of employment has received stock options exercisable for, and

other stock awards of, ordinary shares of Jazz Pharmaceuticals, R. Hume Employee of: Jazz Pharmaceuticals; in the course of employment has received stock options exercisable for, and other stock awards of, ordinary shares of Jazz Pharmaceuticals, A. Hannah Personal Interest: Consultant to Jazz Pharmaceuticals, R. Soiffer Personal Interest: Served on advisory committees with Jazz Pharmaceuticals.

### O131

#### Low levels of insulin-like growth factor 1 and insulin-like growth factor binding protein 3 are associated with an increased risk of veno-occlusive disease after allogeneic HSCT

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**Introduction:** Allogeneic myeloablative haematopoietic stem cell transplantation (HSCT) is challenged by severe adverse events including direct cytotoxicity of the conditioning and systemic inflammatory reactions caused by leaking epithelial barriers. Investigation of factors involved in protection and regeneration of damaged tissues may help reduce treatment-related complications such as acute graft-versus-host disease (aGvHD) and veno-occlusive disease (VOD).

Insulin-like growth factor 1 (IGF-1) has important anabolic actions and contributes to proliferation and differentiation of various soft tissues, in particular epithelial cells. Its binding protein 3 (IGFBP-3) promotes these effects by prolonging the half-life of IGF-1 and by modulating transport of IGF-1 to target tissues. We hypothesised that reduced levels of IGF-1 and IGFBP-3 may lead to increased systemic inflammation and toxic side effects in relation to HSCT.

**Material (or patients) and methods:** 41 patients (age 16.5–55.4 years) undergoing HSCT at Rigshospitalet, Copenhagen were included. Conditioning regimens included total body irradiation plus cyclophosphamide ( $n=34$ ), busulphan plus cyclophosphamide ( $n=6$ ) and fludarabine plus cyclophosphamide ( $n=1$ ). Plasma levels of IGF-1 and IGFBP-3 were measured by chemiluminescence at baseline and at day 0, +7 and +21 post-transplant and were converted into age-standardised values (standard deviation scores, SDS). C-reactive protein (CRP) was measured weekly, and interleukin-6 (IL-6) was measured at day +7 following HSCT. VOD was defined according to the Seattle criteria as  $>2\%$  weight gain from baseline and serum bilirubin  $>34 \mu\text{mol/L}$  occurring within 20 days of transplantation.

**Results:** IGF-1 rose from a baseline value of -0.22 SDS (mean, 95% CI: -0.67–0.24) to a peak of 0.67 SDS (0.22–1.12,  $P=0.0002$ ) at day 0 and then gradually declined. In contrast, IGFBP-3 decreased from a baseline value of 0.004 SDS (-0.46–0.46) towards a minimum of -1.1 SDS (-1.58–[-0.62],  $P=0.0001$ ) at day +21.

IGF-1 levels were inversely correlated with CRP at day 0 ( $r=-0.44$ ,  $P=0.004$ ) and day +7 ( $r=-0.39$ ,  $P=0.011$ ), and similar associations with CRP were found for IGFBP-3 ( $r=-0.70$ ,  $P<0.0001$  and  $r=-0.62$ ,  $P<0.0001$ ). Furthermore, both IGF-1 and IGFBP-3 correlated inversely with IL-6 levels at day +7 ( $r=-0.40$ ,  $P=0.012$  and  $r=-0.61$ ,  $P<0.0001$ ).

Patients who did not develop VOD had higher levels of IGF-1 and IGFBP-3 at baseline compared to patients with VOD ( $n=13$ ) (OR=0.20 per 1 SDS increase in IGF-1 (95% CI: 0.06–0.69),  $P=0.011$  and OR=0.20 (0.05–0.73),  $P=0.015$ , respectively) and during the first week post-transplant (day 0: OR=0.45 (0.22–0.94),  $P=0.032$  and OR=0.44 (0.21–0.94),

$P=0.033$ ; day +7: OR=0.35 (0.15–0.83),  $P=0.018$  and OR=0.17 (0.05–0.59),  $P=0.006$ ) in multivariate analyses. Notably, pre-transplant liver parameters were not associated with IGF-1, IGFBP-3 or VOD. In addition, high levels of IGF-1 at day +21 were associated with decreased occurrence of grade 3–4 aGvHD ( $n=9$ ) compared to grade 0–2 aGvHD (OR=0.46 (0.21–0.98),  $P=0.046$ ) in multivariate analysis.

**Conclusion:** Low levels of IGF-1 and IGFBP-3 before HSCT may be an independent predictor of VOD, irrespective of the liver function at this stage, possibly due to a reduced inflammatory response in patients with high levels of IGF-1 and IGFBP-3.

**Disclosure of Interest:** None declared.

### O132

#### Nationwide Survey of Defibrotide and Recombinant Human Soluble Thrombomodulin for Treatment of Sinusoidal Obstruction Syndrome After Allogeneic Hematopoietic Stem Cell Transplantation

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**Introduction:** Sinusoidal obstruction syndrome (SOS) is a lethal complication following haematopoietic stem cell transplantation (HSCT). British guidelines only recommend defibrotide (DF) for treating SOS (Dignan, Br J Haematol 2013). Recombinant human soluble thrombomodulin (rhTM) is reportedly to be possibly effective for SOS and has been approved in Japan for disseminated intravascular coagulation, which resembles SOS pathologically. We conducted a retrospective nationwide survey of DF and rhTM treatment of SOS in Japan.

**Material (or patients) and methods:** The retrospective analysis included 65 patients who developed SOS after allogeneic HSCT and were treated with DF ( $n=24$ ) or rhTM ( $n=41$ ) in 20 centres during 1999-2011. The median age at transplantation was 40 years (range, 0-69). The diagnoses included acute myeloid leukaemia ( $n=21$ ), acute lymphoblastic leukaemia ( $n=14$ ), myelodysplastic syndrome ( $n=8$ ), lymphoma ( $n=11$ ), chronic myeloid leukaemia/myeloproliferative neoplasm ( $n=6$ ), and others ( $n=5$ ). 19 patients were transplanted from a related donor (bone marrow,  $n=9$ ; peripheral blood,  $n=10$ ) and 46 were transplanted from an unrelated donor (bone marrow,  $n=34$  and cord blood,  $n=12$ ). Graft-versus-host disease prophylaxis was cyclosporine-based ( $n=20$ ) or tacrolimus-based ( $n=44$ ). Conditioning regimens were myeloablative ( $n=45$ ) or reduced-intensity ( $n=20$ ). SOS was defined following the Seattle (McDonald, Hepatology 1984), Baltimore (Jones, Transplantation 1987), or other criteria. Severe SOS was defined as SOS with renal and/or respiratory failure at diagnosis (Yakushijin, BMT 2015). Complete response (CR) for SOS was defined as resolution of all signs and symptoms of SOS diagnostic criteria.

**Results:** Of 56 patients who met the Seattle criteria, 12 also met the Baltimore criteria. The remaining 9 patients were clinically diagnosed. The median doses and durations were 24 mg/kg (range, 7-80) and 15 days (range, 1-46) for DF, and 380 U/kg (range, 130-490) and 8 days (range, 3-52) for rhTM. The 100-day CR rates were 50% and 54% in the DF and rhTM groups, respectively. Fourteen patients had severe SOS (DF group,  $n=3$ ; rhTM group,  $n=11$ ), of whom 4 achieved CR (DF group,  $n=0$ ; rhTM group,  $n=4$ ). Following the Common Terminology Criteria for Adverse Event (version 4), several grade 3+ haemorrhagic adverse events were directly related to DF or rhTM: 1 patient in the DF group experienced both gastrointestinal (Gr3) and pulmonary (Gr4) bleeding, and 5 patients in the rhTM group experienced gastrointestinal (Gr3,  $n=2$ ), bronchial (Gr3), oral (Gr3), or intracranial (Gr4) bleeding. The 100- and 180-day overall survival rates were 50% and 50% in the DF group, and 48% and 38% in the rhTM group.

**Conclusion:** Our results suggest that DF appears to be effective also in the Japanese population. Although rhTM prompted some haemorrhagic adverse effects, it might be an alternative option for SOS. Prospective research is needed to determine the efficacies of these agents.

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### O133

#### Sinusoidal obstruction syndrome in allogeneic hematopoietic stem cell transplantation after prior Gemtuzumab Ozogamicin treatment: an EBMT retrospective survey

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**Introduction:** Many concerns exist about the risk of sinusoidal obstruction syndrome (SOS) when using gemtuzumab ozogamicin (GO) prior to allogeneic stem cell transplantation (allo-HSCT), especially when this is performed within a short interval from the last GO dose ( $\leq 3.5$  months). Our analysis aimed to assess the incidence of SOS after allo-HSCT and the outcomes of a group of AML adults receiving prior GO treatment.

**Material (or patients) and methods:** SOS was classified as possible, probable or definite according to the presence of Seattle and/or Baltimore clinical criteria and/or according to time to onset. The primary endpoint was the assessment of SOS incidence. Secondary endpoints were engraftment, non-relapse mortality (NRM), graft-versus host disease (GVHD), leukemia-free survival (LFS) and overall survival (OS).

**Results:** A total of 146 patients (71 women and 75 men) was identified. Median age was 50 years (range 19-70). In most cases ( $n=127$  out of 137, 93%) GO was administered in association with other chemotherapeutic agents. Median GO dose was 3 mg/m<sup>2</sup> (range 3-9). Median number of cycles before allo-HSCT was 1 (range 1-6). Most patients ( $n=79$ , 54%) received GO as induction treatment, while 51 (35%) and 16 (11%) for relapsing or primary refractory disease, respectively. At time of allo-HSCT 97 (66%) patients were in complete remission. Of note, 11 patients underwent prior allo-HSCT and 8 patients prior auto-HSCT. Most patients received a reduced intensity conditioning (RIC) regimen ( $n=84$ , 58%). Despite prior GO treatment, only sixty-nine patients (45%) received SOS prophylaxis during allo-HSCT (heparin  $n=57$ , ursodeoxycholic acid  $n=8$ , and defibrotide  $n=4$ ). Cumulative incidence of SOS was 8% ( $n=11$ ), with 4 cases (5%) in patients receiving RIC as compared to 7 (11.5%) in patients receiving MAC ( $P=0.056$ ). SOS was the main cause of death in 3 cases. Median interval between last GO dose and allo-HSCT was 130 days (range 13-1126). No difference in OS or SOS incidence was found when comparing patients receiving GO shortly before allo-HSCT ( $\leq 3.5$  months) to the others. None of the analyzed risk factors had an impact on the risk of SOS in our study. With a median of 15 days, CI of neutrophil and platelet engraftment was 94% and 89%, respectively. CI of acute GVHD at day 100 was 31%, occurring in 84 patients (out of them 45 had grade II-IV GVHD), while CI of chronic GVHD at 5 years was 25%. Median follow-up was 64 months. Probability of OS and LFS at 5 years was 40% and 37%, respectively.

Cumulative RI and NRM at 5 years were 42% and 21%, respectively. In multivariate analysis LFS and OS were worse in patients transplanted with an active disease ( $P < 0.03$ ) and OS was worse in patients having presented hyperbilirubinemia or transaminases elevation during GO treatment ( $P < 0.03$ ).

**Conclusion:** Our results in a large cohort of AML patients show that GO administration prior to allo-HSCT is associated with an SOS incidence similar to previous reports for patients undergoing allo-HSCT without prior GO treatment. Interestingly, a short interval between the last dose of GO and allo-HSCT was not confirmed as being associated to a higher incidence of SOS in our population. Moreover, prospective studies investigating the role and the utility of SOS prophylaxis are warranted.

**Disclosure of Interest:** None declared.

#### O134

##### **Altered Defibrinolytic treatment regimen for Venous-occlusive disease: A single center experience**

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**Introduction:** Venous-occlusive disease (VOD), also known as sinusoidal-obstructive syndrome is a well-defined clinical syndrome, usually manifested clinically by painful hepatomegaly, fluid retention and hyperbilirubinemia. Current treatment of VOD includes adequate fluid and sodium balance, and diuretics. In severe VOD, presented as multi-organ failure (MOF) or bilirubin above 8mg/dl, Defibrinolytic (DF) for 14-21 days is recommended. The aim of the study is to explore whether DF treatment time of moderate/severe VOD can be shortened, based on the resolution of disease signs?

**Material (or patients) and methods:** A retrospective analysis of transplanted patients between January 2011 and April 2015 who were diagnosed with moderate to severe VOD and treated with DF. VOD diagnosis was based on Baltimore or modified Seattle criteria. Fifteen patients having moderate VOD who failed on high doses of diuretics, or those suffering from severe VOD as defined above, were given DF at a dose of 25mg/kg/d. Eleven out of 15 patients were given DF for a period of less than 14 days.

**Results:** Two of 11 patients developed VOD following autologous stem cell transplantation (SCT), two following matched related SCT, and seven following MUD SCT. Using either Baltimore or modified Seattle criteria, four patients were defined as having moderate VOD and seven as having severe VOD with renal, lungs or central nervous system involvement. Median patient age was 37 (0.5-70) years, 6 patients underwent SCT for leukemia, 2 for Neuroblastoma, 1 for myeloma, 1 for aplastic anemia and 1 for SCID. VOD developed on a median of 14 days post stem cell reinfusion (range 8-16). Median weight gain was 18% above base line (range 3.7-28%). Ascites was evident in 10 of 11 patients, and in 6 of 11 it was moderate-severe. Median bilirubin was 4.1 (range 0.7-13.8). DF was given for a median period of 8 days (range 3-13d) and was discontinued when bilirubin was significantly decreased (below 2mg/dl), weight gain reversed to no more than 5% above baseline, or a resolution of above mentioned MOF symptoms occurred. Ten out of 11 patients achieved full resolution of VOD. One patient died from sepsis and respiratory failure a few days after DF was stopped because of suspected pulmonary hemorrhage.

**Conclusion:** Shorter duration (<14d) with DF is feasible. Decision-making regarding DF treatment duration should be guided by resolution of VOD symptoms, rather than a rigid time frame. A larger cohort is needed to consolidate the above results.

**Disclosure of Interest:** None declared.

#### O135

##### **Alterations of plasma complement C3b, C5b and VWF, ADAMTS13 in patients with thrombotic microangiopathy after hematopoietic stem-cell transplantation**

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**Introduction:** Transplant-associated microangiopathy (TMA) is an uncommon but devastating complication in patients undergoing hematopoietic stem-cell transplantation (HSCT). It may be confused with severe graft-versus-host disease (GVHD), infection, and other transplant related thrombotic diseases. Limited studies have shown the changes in plasma VWF/ADAMTS13 or complement activation markers in patients after HSCT. However, the role of VWF/ADAMTS13 and complement activation in patients with TMA and GVHD is not fully understood. Current study is to investigate the alterations of plasma levels of C3b, C5b and VWF/ADAMTS13 in patients with TMA and to explore their roles in the pathogenesis and early diagnosis of transplant-associated TMA.

**Material (or patients) and methods:** From 2011 to 2014, 14 patients with TMA were enrolled into the study in a single medical center. 71 other patients following HSCT were recruited as control subjects including 11 cases of hepatic vein occlusion disease (VOD), 20 cases of severe infections, 20 cases of severe II-IV<sup>o</sup> GVHD and 20 cases without complications. Blood sample was collected before transplantation and at the onset of transplantation related complication. Fluorescence resonance energy transfer substrate (FRETs)-VWF73 assay detected plasma ADAMTS13 activity. Collagen-binding assay and latex immunoassay determined VWF activity and VWF antigen, respectively. Plasma VWF multimer was determined by agarose gel electrophoresis and Western blot. Plasma levels of complement C3b and C5b were measured with ELISA.

**Results:** Compared with the levels before transplantation, plasma ADAMTS13 activity and VWF antigen or activity in the patients with TMA did not differ from those who developed TMA, with infection or GVHD or without any complication ( $P > 0.05$ ). However, plasma ADAMTS13 activity decreased and the ratio of VWF antigen/activity increased significantly in patients with VOD ( $P < 0.05$ ). Plasma VWF multimer distribution was similar in patients with infection, GVHD or without complication, but ultralarge multimers of VWF was present in patients with TMA and VOD. Plasma levels of complement C3b was increased in patients after HSCT (198.46 ng/ml  $\pm$  14.78 ng/ml) compared with healthy subjects (85.02 ng/ml  $\pm$  8.50 ng/ml) ( $P < 0.05$ ), but exhibited no difference in the other groups. Plasma C3b increased significantly in patients with TMA and GVHD ( $P < 0.05$ ). The plasma levels of C3b were higher in the TMA group (480.70 ng/ml  $\pm$  66.76ng/ml) than the GVHD group (298.50 ng/ml  $\pm$  32.06 ng/l) ( $P < 0.05$ ). Also, plasma levels of C5b in patients with TMA were significantly increased (1059.49 ng/ml  $\pm$  85.57 ng/ml) as compared with those before transplantation (653.19ng/ml  $\pm$  44.91ng/ml) and other groups ( $P < 0.05$ ).

**Conclusion:** We conclude that plasma ADAMTS 13 activity and the ratio of VWF antigen/activity remained stable in the patients with transplant-associated TMA, but the levels of complement C3b and C5b, particularly the C5b, increased significantly, suggesting the critical role of complement pathway in the pathogenesis of TMA. C5b may be a specific biomarker for early diagnosis of TMA but C3b is a marker for both TMA and GVHD.

**Disclosure of Interest:** None declared.

O136

**Identification and prognosis impact of intestinal thrombotic microangiopathy after allogeneic stem cell transplant**

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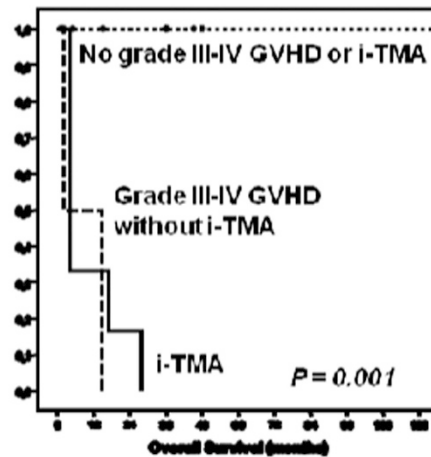
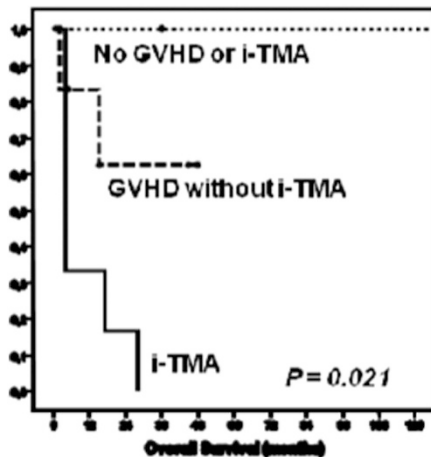
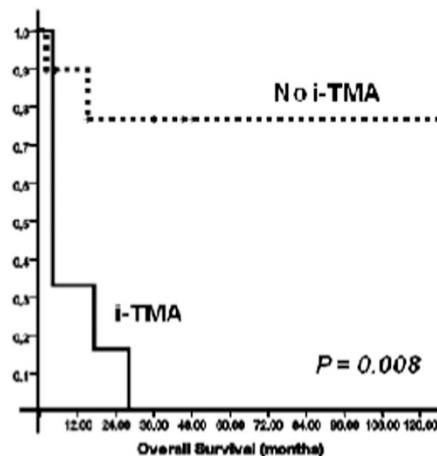
**Introduction:** Transplant-associated thrombotic microangiopathy (TA-TMA) can manifest as a multi-system disease occurring after various triggers of small vessel endothelial injury, leading to subsequent tissue damage in different organs. While the kidney is most commonly affected, TA-TMA involving organs such as the lung, bowel, heart, and brain is now known to have specific clinical presentations. In fact, there is growing evidence that highlighting the impact of TA-TMA on the gastrointestinal tract after hematopoietic stem cell transplant (HSCT). However, similar gastrointestinal symptoms may also be findings of acute gut graft versus host disease (GVHD), making the clinical diagnosis difficult. Moreover, identification of intestinal TA-TMA (i-TMA) is very important since continuation of calcineurin inhibitors can significantly worsen symptoms in affected patients, which is an opposite treatment for acute GVHD. Therefore, unidentified i-TMA can lead to significant morbidity and mortality. Our aims were to identify those patients misdiagnosed with i-TMA, and

to determine the clinical impact of i-TMA on this series of patients.

**Material (or patients) and methods:** We retrospectively analyzed data from all allogeneic HSCT recipients, transplanted between 2004 and 2012, who were evaluated with colonoscopy, including mucosal biopsies, in our hospital. Clinical symptoms of i-TMA and laboratory findings of TA-TMA were recorded. Other post-HSCT complications such as GVHD were also recorded. Retrospective colonoscopic examinations were performed. All biopsy specimens of the gut were retrospectively examined.

**Results:** In this period of time, 322 patients (≥18 years old) received an allogeneic HSCT. The incidence of grade III-IV acute GVHD and systemic TA-TMA were 15.5% and 6.8%, respectively. A colonoscopy was performed in 30 patients, and 21 biopsies of 17 patients were evaluated. The main indications for colonoscopy were an undiagnosed gastroscopy and persistence of a high suspect of acute GVHD, the presence of intestinal bleeding or suspicion of viral infection. Six out of 17 evaluable patients (35%) were retrospectively diagnosed with i-TMA. All 6 patients were initially diagnosed with acute GVHD. Only 3 out of 6 i-TMA patients had systemic TA-TMA according to probable TA-TMA criteria by Cho, *et al* 2010, but none of these 3 patients fulfilled the previously established international criteria (Ho, *et al* 2005, Ruutu, *et al* 2007). Most important, i-TMA was associated with a worse outcome. All patients with i-TMA were death at 28 months, whereas median overall survival was not reached for patients without i-TMA ( $P=0.008$ ). Moreover, patients retrospectively diagnosed with i-TMA had a worse outcome than those with acute GVHD (and without i-TMA) ( $P=0.021$ ). Finally, no difference in overall

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survival was observed between patients with i-TMA compared with those with grade III-IV acute GVHD (Figure 1).

**Conclusion:** Intestinal TA-TMA is a misdiagnosed complication of allogeneic HSCT with a high mortality rate. Therefore, a clinical suspect became crucial for diagnosis of these patients. Probably, an early clinical diagnosis of i-TMA, with the prompt initiation of an appropriate therapy, could contribute to improve the overall survival of these patients.

**Disclosure of Interest:** None declared.

### O137

#### Active microbiological surveillance improves the management of multidrug resistant gram-negative infections after HSCT: a prospective study in 524 patients

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**Introduction:** The emergence of multidrug resistant gram-negative (MDR-GN) pathogens causing life-threatening infections in haematological cancer patients has become a worldwide challenge due to high mortality and management costs. Therefore, strategies of active surveillance for identification of MDR-GN carriers at risk of subsequent infection during intensive treatments are urgently needed.

**Material (or patients) and methods:** From Jun 2012 – Aug 2015 (38 months), we actively surveilled MDR-GN colonization status through weekly rectal swabs in 524 consecutive hematological patients (286 undergoing allogeneic hematopoietic stem cell transplantation (HSCT), 99 autologous HSCT and 145 receiving chemo or supportive therapy) from the time of hospital admission. Blood cultures were also performed at each febrile episode. Patients were treated according to internal antimicrobial guidelines, usually with multiple targeted antimicrobial therapy in case of a previously known MDR-GN colonization.

**Results:** Overall, 4168 rectal swabs were collected, 429/4168 (10%) showing positivity in 152/524 (29%) patients. Distribution of MDR-GN colonization was as follows: carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) 43 (23%) cases, extended-spectrum beta lactamase producing (ESBL) *E.coli* 43 (23%), *Pseudomonas aeruginosa* 41 (21%), *Stenotrophomonas maltophilia* 39 (20%), ESBL+ *Klebsiella pneumoniae* 2 (1%), other species 23 (12%). Carriers of MDR-GN experienced a higher number of bloodstream infections compared to non-colonized patients ( $p < 0.0001$ ) and were subjected to higher number of swabs (average  $10,4 \pm 8,3$  SD versus  $7,3 \pm 6,2$  SD;  $P=0.0006$ , respectively). While in non-colonized patients, only 4/372 (1%) developed a MDR-GN sepsis, this percentage raised up to 29% (45/152) in case of colonization ( $P < 0.0001$ ). Distribution of MDR-GN infections was as follows: KPC-Ko 15 (31%) cases, *E. coli* ESBL+ 13 (27%), *P. aeruginosa* 9 (18%), *S. maltophilia* 6 (12%), *K. pneumoniae* ESBL+ 3 (6%), other species 3 (6%).

The risk for MDR-GN infection was higher in allogeneic HSCT (66% of cases) decreasing in chemo- or supportive therapy (20%) and finally in the autologous HSCT (14%) group. Despite aggressive treatment, overall mortality was 33% (16/49), being attributed to KPC-Kp in 8 (50%) cases, *S. maltophilia* 4 (25%), *P. aeruginosa* 3 (19%) and to *E. Coli* ESBL+ 1 (6%).

In allogeneic HSCT, pre-transplant colonization status was evaluable for 236/286 pts, 51/236 (22%) of which were transplanted regardless a known MDR-GN positivity. Only 8/51 (16%) developed a subsequent infection from the same MDR-GN pathogen, with an attributed mortality of 25%. Moreover in 18/49 (37%) patients, a positive rectal swab

anticipated sepsis with a median range from positivity to the onset of infection of 38,5 days (range 3-222).

**Conclusion:** Positive rectal swabs significantly associate, and potentially anticipate, the development of a subsequent MDR-GN infection. Data from active surveillance and a timely application of prophylactic (i.e contact precautions, intensified hygienic measures in carriers) and therapeutic strategies (i.e prompt targeted therapy) may reduce infectious mortality in hematological cancer patients. Moreover, MDR-GN colonization before allogeneic HSCT should not preclude clinicians from proceeding to transplant.

**Disclosure of Interest:** None declared.

### O138

#### An emerging opportunistic infection: fatal animal-astrovirus encephalitis in a paediatric stem cell transplant recipient

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**Introduction:** Neuroinvasive astrovirus is an emerging life-threatening infection in immunocompromised hosts. We describe a 8-month-old child who died of fatal animal astrovirus encephalitis following a haematopoietic stem cell transplant for acute myeloid leukaemia (AML) with high-risk cytogenetics, t (10;11) in first remission.

**Material (or patients) and methods:** The child achieved remission following ADE therapy and received two courses of consolidation with FLAG-IDA. The treatment was complicated by *Enterobacter* neutropenic fevers and enteritis due to human astrovirus infection, followed by persistent asymptomatic astrovirus detection in her stools. HLA-matched, major ABO-mismatched unrelated donor bone marrow transplant was done following conditioning regimen with busulfan, cyclophosphamide, melphalan and alemtuzumab and post-transplant graft-versus-host prophylaxis with ciclosporin. Neutrophil engraftment was demonstrated on Day +17 and donor chimerism was 100%. Early transplant course was complicated by culture negative neutropenic fevers, veno-occlusive disease and grade 1 cutaneous graft-versus-host disease that responded to topical steroid. Nutrition rehabilitation was complicated by protracted human astrovirus positive diarrhea, which was finally resolved upon discharge home on Day +94. Her stool was astrovirus negative on Day +92. Her ciclosporin was tapered off by Day +100.

**Results:** The child became encephalopathic at Day +120 and subsequently developed uncontrolled dystonic movement. Cerebrospinal fluid analysis done on two occasions showed albuminocyttoplasmic dissociation and real-time PCR was negative for herpesviridae (herpes simplex virus I and II, varicella zoster, cytomegalovirus, Epstein-Barr virus, HHV6, and HHV7), papovaviridae (BK, JC) adenovirus, enteroviruses, parechovirus, norovirus, human astrovirus, measles, and Toxoplasma. Cerebrospinal fluid culture did not yield any positive results. Repeat brain images indicated progressive brain volume loss, very poor myelination and high signal within bilateral basal ganglia. Brain biopsy showed non-specific necrotic neurons with featureless nuclei and pyknotic cells. Despite receiving a top up donor marrow to facilitate immune reconstitution, the child died of irreversible global brain dysfunction on Day +196. Encephalitis due to Astrovirus HAstV-VA1/HMO-C-UK1 was diagnosed based on deep sequencing of post-mortem brain biopsy tissue. This astrovirus belongs to VA1 and HMO-C group of astroviruses and is associated with neurological illnesses in mink and cattle.

**Conclusion:** Our patient is the third paediatric patient with astrovirus HAstV-VA1/HMO-C-UK1 encephalitis and only 6 human infections with this virus have been reported to date.

The diagnosis of animal-astrovirus encephalitis is challenging and these cases illustrate the value of including testing for astrovirus HAstV-VA1/HMO-C-UK1 in the diagnosis of encephalitides in immunosuppressed patients. We suggest that deep sequencing for this virus should be performed promptly in an immunodeficient host with unexplained encephalopathy.

**Disclosure of Interest:** None declared.

### O139

#### **Modulation of HHV-6B cell receptor OX40 (CD134) expression on CD4+ T cells may favour HHV-6B infection after allogeneic cord blood transplantation**

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**Introduction:** HHV-6B reactivation after cord blood transplantation (CBT) is documented in more than 80% of cases. Mechanisms underlying such infections remain unclear. Recently, OX40 (CD134), a member of the TNF receptor superfamily expressed on activated T lymphocytes, has been identified as a specific HHV-6B entry receptor. We hypothesized that overexpression of CD134 on CB grafted cells or during immune recovery after CBT could favor HHV-6B infection.

**Material (or patients) and methods:** This prospective study aimed at comparing CD134 expression on CD3+/CD4+ T-cells first between CB and peripheral blood stem cells (PBSC) grafts, then during early immune recovery, after CBT or PBSCT on blood samples collected at day 0, then every 15 days up to day +60 post-transplant after informed consent. FITC-conjugated CD134 (BD Bioscience, San Jose, CA) was used to assess CD134 expression in multiparameter flow cytometry (Navios, Beckman Coulter, Miami, FL) by comparison to an isotypic control. A mean fluorescence intensity ratio of 2 or higher was considered significant. HHV-6B reactivation was assessed by standard viral PCR on the same samples.

**Results:** Between December 2014 and June 2015, CD134 expression was analyzed on 11 CB grafts from 9 allotransplanted children (median age 4 years (range 2-19), males 33%, lymphoid disease 44%, complete remission (CR) at transplant 67%, single CBT  $n=7$ ; double CBT  $n=2$ , myeloablative regimen 78%) and 12 PBSC grafts from 13 allotransplanted adults (median age 63 years (range: 43-70), reduced conditioning regimen 100%, donor type sibling  $n=6$ , matched unrelated  $n=3$ ; haplo-identical  $n=4$ ).

While all PBSC grafts contained CD134+/CD4+ cells, this was the case for only 3 CB grafts. Median CD134+/CD4+ cell percentages were significantly higher among PBSC (7.91% range 0.04-21.53) compared to CB (1.14% range 0.31-5.89,  $P=0.01$ ).

After transplant, CD4+ T-cell recovery, until day +30, was slower after CBT than PBSCT (respectively medians at day +15  $1/\text{mm}^3$  (0-36) vs  $13/\text{mm}^3$  (1-86)  $P=0.01$ ; +30  $11/\text{mm}^3$  (0-106) vs  $42/\text{mm}^3$  (5-736)  $P=0.07$ ). Samples tested CD134+/CD4+ were similar between CBT (25/27 92.5%) and PBSCT (54/54 100%,  $P=0.1$ ) and such as soon as day+15. Overall, the median percentages of CD134+/CD3+ T-cells were higher after CBT (18,99%, range: 2-38.6%) than after PBSCT (10,55%, range 1.2-35%;  $P=0,04$ ).

HHV-6B positive PCR was documented in 10/22 patients and 23/100 samples after transplant. The median viral load was 3 log UI/mL (range 2-5.4). HHV-6 positive PCR was asymptomatic in all but one CBT patient who died at day +32 from HHV-6B pneumonia. As expected, the numbers of HHV6+ samples were significantly higher after CBT than PBSCT (16/40 vs 7/60  $P=0.001$ ) with a peak at day+30 (86% of positive samples vs

23% for PBSC group). There was a trend for higher median percentage of CD134+/CD4+ T-cells in the HHV-6+ group (32.4% (8.51-81.25) vs 28.8% (1.48-59.89),  $P=0.05$ ).

**Conclusion:** Expression of the viral receptor CD134 on naïve CB T-cells increases during immune reconstitution. This expression is higher than after PBSCT and precedes HHV-6B peak infection after CBT, suggesting no role for HHV-6B regarding CD134 induction on CB CD4+ T cells. Thus, modulation of CD134 on CD4+ T cells may favor HHV-6B infection after CBT. Use of an anti-CD134 antibody, could be considered to alleviate the risk of HHV-6 infection in this context.

**Disclosure of Interest:** None declared.

### O140

#### **Risk factors and consequences of CMV and EBV reactivation after TCR alpha/beta and CD19+ depleted unrelated and haploidentical HSCT in pediatric patients**

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**Introduction:** The reactivation of CMV and EBV after hematopoietic stem cell transplants is associated with significant morbidity and mortality. Most methods of T-cell depletion increase the risk of virus reactivation, but the data remain scarce for the TCR alpha/beta and CD19+ depletion.

**Material (or patients) and methods:** In this study were included 182 patients after haploidentical and unrelated HSCT with TCR alpha/beta and CD19+ depletion performed during 2012-2014. Patients after second transplants, patients with active viral replication at the moment of HSCT and those who received prophylactic post transplant cell therapy with CD45RA-depleted DLI were excluded. Male:female ratio was 124:58, 114 had malignant and 68 nonmalignant diseases, median age was 6,4 y. One hundred twenty four transplants were from unrelated (9/10 or 10/10 matched) and 58 from haplo donors. Donor/recipient serostatus for CMV was: D+/R- in 13, D+/R+ in 77, D-/R+ in 51, D-/R- in 17, unknown for 1 donor, 23 patients with combined PIDs were excluded due to uninterpretable serostatus. Conditioning regimens contained treosulfan and fludarabine+/-melphalan for malignant and PID patients and were Cy/Flu-based for aplastic anemia, 100 patients received serotherapy with horse ATG (ATGAM), 41 patient received thymoglobulin+rituximab. CMV and EBV DNA were monitored weekly by RQ PCR. CMV viremia was registered if more than 500 copies per ml of blood were detected. In all cases of CMV positivity patients received standard preemptive therapy with gancyclovir.

**Results:** The cumulative incidence of CMV reactivation was 0,51 (95% CI 0,44-0,6). Median time to reactivation was 5 weeks. Median reactivation duration was 3 weeks. In univariate analysis two factors increased the CI of CMV reactivation - acute GVHD >grade II, (0,67 vs 0,4  $P=0,003$ ) and the diagnosis of malignant vs non-malignant disease (0,58 vs 0,39,  $P=0,028$ ). In CMV-negative recipients CI of CMV reactivation was 0,36 for CMV- donor and 0,3 for CMV+ donor vs 0,56 in CMV D+/R+ and 0,57 D-/R+ ( $P=0,25$ ). Age, sex, type of donor, type of serotherapy did not significantly affect CMV reactivation rates. The incidence of CMV disease was 0,049. CMV reactivation did not significantly affect the incidence of relapse and TRM in patients with malignant disease. No correlation was found between CMV viremia and the tempo of the immune recovery of CD3+, CD16+56+ and TCR gamma/delta cells on days +30 and +60.

The cumulative incidence of EBV reactivation was 0,33 (95% CI 0,26-0,42). Median time to reactivation was 13,5 weeks.

Median reactivation duration was 2 weeks. Factors influencing EBV reactivation were GVHD, 0,43 vs 0,27 in patients with/without aGVHD grade 2-4 ( $P=0,02$ ), administration of rituximab in conditioning, 0,24 and 0,36 in groups with and without rituximab, respectively ( $P=0,124$ ). Five patients received preemptive therapy with rituximab to control high EBV load or mononucleosis symptoms. There were no cases of PTLD. Overall survival was not dependent on CMV and EBV reactivation.

**Conclusion:** TCR alpha/beta and CD19+ depletion in HSCT is not associated with excess of CMV reactivation and disease and resolves the problem of PTLD as life-threatening posttransplant complication. Reactivation of CMV and EBV are not associated with adverse outcomes of the procedure.

**Disclosure of Interest:** None declared.

#### O141

##### Dynamics of humoral immunity against measles, mumps and rubella post allogeneic hematopoietic cell transplantation

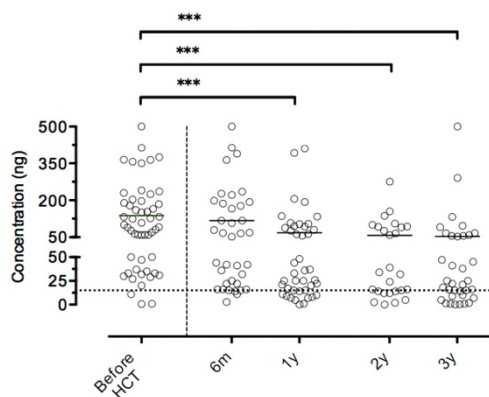
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**Introduction:** Following allogeneic hematopoietic cell transplantations (HCT) the immune system is severely compromised due to pharmacological immunosuppression and graft-induced disruption of the lymphoid tissues by the conditioning and the allograft. According to well-established guidelines re-vaccinations against a range of pathogens are recommended, however, live-vaccines in general should be avoided in the early post-HCT period. Due to the longevity of plasma cells and their antibodies some protective titers can be sustained even after HCT; the contribution of donor B and plasma cells to antibody production has not been clearly elucidated.

**Material (or patients) and methods:** We analyzed serial antibody titers in 79 consecutive patients who underwent allogeneic HCT and their HLA-matched related donor (MRD) between 2009 and 2014 at our single center. Antibody titers against measles, mumps and rubella were measured prior to HCT, at 6 months (m), 1, 2, and 3 years (y) post-HCT.

**Results:** The majority of donors had protective antibody levels against measles (97%), mumps (96%), and rubella (93%). Likewise, most recipients had sufficient antibody titers (measles 94%, mumps 85%, rubella 92%). Including only recipients of seropositive donors analysis revealed declining antibody titers post-HCT: while antibody titers against measles were rather stable and protective in 94% of patients @ 6m, 90% @ 1y, 95% @2y, and 79% @ 3y post-HCT those against mumps decreased promptly with protective titers in 77% @6m, 75% @1y, 50% @2y and 47% @3y post-HCT. Similarly, antibody levels against rubella declined during the first year post-HCT and remained rather stable thereafter (protective levels in 82% of patients @6m, 69% @1y, 76% @ 2y, and 67% @3y). Fig. 1 displays the corresponding absolute anti-rubella



titers. Interestingly, we observed that 77% of patients given mobilized peripheral blood but none of the 5 bone marrow (BM) recipients had protective anti-rubella serotiters @1y post-HCT ( $P < 0,01$ ).

**Conclusion:** Antibodies are reported to have a half-life of approximately 80 days. Our data show that despite donor seropositivity antibody titers significantly decline after allo-TPL. Whether newly synthesized antibodies beyond 1y post-HCT derive from residual host or donor plasma cells needs to be clarified. Rather unexpected is the observation that BM grafts – despite a higher plasma cell content – appear to provide less antibody protection post-HCT, underlining the potential protective role of the residual host compartment. Clarification of the origin of antibodies post-HCT can help to establish donor and host vaccination strategies prior to HCT.

**Disclosure of Interest:** None declared.

#### O142

##### Validation of a risk score for post-engraftment invasive fungal disease in 414 adult allogeneic hematopoietic stem cell transplant recipients

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**Introduction:** Recently, we reported a simple prognostic score for post-engraftment invasive fungal disease (IFD) in 404 adult allogeneic hematopoietic stem cell transplant (alloSCT) recipients using 5 pre- and post-transplant variables, which could be useful to improve prophylactic strategies in the future. Our prognostic score model classified alloSCT recipients into three groups. The low-risk group showed 0.7% cumulative incidence (CI) of probable/proven IFD, while in the intermediate- and high-risk groups it was 9.9% and 24.7%, respectively. Nevertheless, the post-engraftment IFD score should be validated in an external cohort.

We aim to validate the post-engraftment IFD score in an external cohort of adult patients submitted to alloSCT.

**Material (or patients) and methods:** Patients with hematological malignancies undergoing an alloSCT were eligible if they fulfilled all the following criteria: 1) stable myeloid engraftment; 2) patients surviving more than 40 days after alloSCT; and 3) patients without previous suspicion or diagnosis of IFD during the pre-transplant or early post-transplant period. From January 2000 to June 2015, 414 consecutive adult patients were included at the Hospital Universitario Reina Sofía ( $n=278$ ) and Hospital Universitario y Politécnico La Fe ( $n=136$ ). Patients were transplanted from a sibling or an unrelated donor, including cord blood (UCBT), according to the donor availability and alloSCT timing. According to the institutional policies of IFD prophylaxis, unrelated or mismatched alloSCT patients received an oral triazole from engraftment until day +90-100, while sibling HLA-identical recipients received oral fluconazole. Diagnosis and classification of IFD was performed according to the EORTC/MSG definitions of 2008. The probabilities of IFD were estimated by the CI method and were compared by the Gray test.

**Results:** Of the 414 patients, 221 (53 %) were male, and the median age was 46 years (range, 14–69 years). The most frequent underlying diseases were acute myeloid leukemia 135 (33%), and acute lymphoblastic leukemia 57 (21%). The majority of patients received alloSCT from HLA identical sibling donor 227 (55%), while 68 (16%) patients underwent a UCBT. Conditioning regimen was myeloablative in 237 (57%). The overall CI of probable/proven IFD was 8.5% at 12 months. The 5 prognostic variables included in the risk-model were distributed as follows: age >40 years (58%),  $\geq 1$  previous SCT (20%), >15 days of pre-engraftment neutropenia (55%), extensive cGVHD (16%), and CMV reactivation until day +180 (46%). Similarly to the previous study, patients were grouped

into low-risk (0-1 points), 138 patients; intermediate-risk (2 points), 149 patients; and high-risk (3-5 points), 127 patients. The CI of IFD at 12 months for low-, intermediate-, and high-risk patients was 2.9%, 8.3%, and 14.7%, respectively ( $P=0.004$ ).

**Conclusion:** To our knowledge, this is the first prognostic index to predict the occurrence of post-engraftment IFD after alloSCT that has been validated in an external cohort. Risk-adapted antifungal prophylactic strategies based on our prognostic index are warranted.

**References:** Montesinos P, Rodríguez-Veiga R, Martínez-Cuadrón D, et al. Treatment of invasive fungal disease using anidulafungin alone or in combination for hematologic patients with concomitant hepatic or renal impairment. *Rev Iberoam Micol.* 2015 Jul-Sep;32(3):185-9.

**Disclosure of Interest:** None declared.

#### O143

##### Monitoring of adenovirus-specific T cells after HSCT in children: equal detection of hexon and penton-specific T cells

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**Introduction:** In pediatric patients, human adenovirus (hAdV) reactivation after hematopoietic stem cell transplantation (HSCT) is frequent and often associated with high morbidity and mortality. Viral clearance is usually achieved by a functional anti-hAdV T-cell response. So far, hAdV hexon has been recognized as a major target of the anti-hAdV immune response. Here, we present data from a prospective study monitoring hexon- and penton-specific T-cell responses in 33 children with hAdV reactivation after HSCT.

**Material (or patients) and methods:** The study was approved by the institutional review board. Patients and legal guardians gave their written informed consent prior to inclusion. All patients undergoing HSCT were routinely monitored for hAdV reactivation in blood and stool by weekly quantitative PCR following institutional guidelines. All patients with quantifiable hAdV in stool or blood entered the T-cell monitoring program. After leukocyte engraftment peripheral blood mononuclear cells (PBMC) were collected once per week. hAdV-reactive T cells were determined by interferon- $\gamma$  (IFN- $\gamma$ ) ELISPOT assay using overlapping peptide pools derived from the hAdV-C5 proteins hexon and penton. Patients were grouped into strong (>40 spots per 250000 PBMC), intermediate (20-40) and weak ( $\leq 20$ ) responders. Control of viral replication was defined as  $\leq 1000$  copies/ml in blood and/or  $< 10^5$  copies/ml in stool. Statistical analysis was carried out using GraphPad Prism 6 software.

**Results:** 33 patients with hAdV reactivation after HSCT were identified between 10/2011 and 10/2015. 16 patients had a malignant disease, 17 patients suffered from non-malignant diseases. In all patients stool PCR was positive for hAdV (median 7 days after HSCT), while 22 patients had hAdV blood virus loads >1000 copies/ml (median 23 days after HSCT). Most patients received standard antiviral treatment with cidofovir (median 4, range 2-11 doses). Only one patient died of hAdV disease; however, in accordance with earlier data one-year overall survival was slightly inferior in patients with >10 000 copies hAdV/ml blood (67% vs. 89%,  $P=0.17$ ). hAdV-specific T cells were first detected at a median of 63 days after SCT with no timely difference between hexon- and penton-reactive T cells. The magnitude of T-cell responses against hexon and penton was individually diverse. While all but one patient demonstrated hexon-reactive T cells, 26 of 31 evaluable patients had T-cells reactive to the penton pool. Six of 31 patients showed strong reactions to penton while 11

of 31 showed strong reactions to hexon. There was no correlation between the target of the immune response and the HLA-type detectable in this small cohort. Control of hAdV replication was achieved in tight association with first detection of hAdV-specific T cells (stool +6.5 [range -18 to +33] days after first detection; blood -1 [-28 to +42] days) demonstrating the importance of hAdV-specific T cells for hAdV control. Hexon and penton-reactive T cells were equally effective in mediating viral control, suggesting that hAdV penton is a second immunodominant target in hAdV infection.

**Conclusion:** hAdV-specific T cells directed against hexon and penton confer protective immunity in HSCT recipients with hAdV reactivation. Extended monitoring of hAdV-specific T cells helps tailoring antiviral treatment and identifies patients suitable for adoptive transfer of hAdV-specific T cells.

**Disclosure of Interest:** None declared.

#### O144

##### Impact of NK cell reconstitution and recipient HLA-C typing on clinical outcome after reduced intensity cord blood transplant: Results Of A Prospective Phase II Multicentric Trial

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**Introduction:** Unrelated cord blood transplantation (UCBT) after reduced intensity conditioning regimen (RIC) has extended the use of UCB in elderly patients and those with co-morbidities without an HLA identical donor. KIR ligand incompatibility between donor and recipient might favor Natural Killer (NK) cell alloreactivity after UCBT in AML patients (Wilhemz et al, 2009). The aim of the present study is to analyze the impact of KIR ligand incompatibilities and NK cell reconstitution on outcome after RIC-UCBT in a prospective trial.

**Material (or patients) and methods:** Seventy-six patients were enrolled for an AML in complete remission in a French prospective trial (Rio, 2015) in 23 centers from Oct. 2007 to Sept. 2009. Peripheral blood samples were collected during the first year following UCBT in order to realize an extensive prospective phenotypic and functional study of NK cells. NK biological data were available at M1 for 54 patients. The inhibitory Killer-Immunoglobulin Receptors (KIR) KIR2DL1, and KIR2DL2/3 bind KIR ligand C2 and C1 respectively, resulting in inhibition of NK-cell mediated lysis. Recipients and UCB were classified into C1 or C2 family depending on their HLA-C typing (C1-C1, C1-C2 or C2-C2).

**Results:** Among the 54 patients, 35 events occurred (relapse or TRM). Median EFS and OS were 13.2 and 18.3 months, respectively. Recipients C2-C2 had a significant worse EFS and OS than C1-C1 or C1-C2 (median EFS C2-C2=3.8 month vs 15.1 month for C1-x;  $P=0.002$ ); median OS C2-C2 3.8 months vs 29.9 months for C1-x; HR=6.12,  $P=0.001$ ). High intracellular staining of CD107a, reflecting the capacity of NK degranulation with HLA negative K562 target, correlated with better OS. CD107a expression was divided in 2 groups at median (=51%). Median OS of CD107 (0-50%) was 12.8 months vs 20.9 months for CD107a (51-66);  $P=0.029$ . Relapse risk was highly increased in recipients C2-C2 (HR=5.04,  $P=0.02$ ). Low expression of CD16 (HR=0.97,  $P=0.043$ ), high expression of HLA-DR (HR=1.08,  $P=8e-04$ ) on NK cells, and recipients C2-C2 (HR=9.44,  $P=0.026$ ) significantly increased the risk of TRM. The inhibitory KIR2DL1 receptor binds to C2 ligands. Of interest, KIR2DL1 was significantly decreased on C2-C2 recipients NK cells at M1, as compared to C1-x recipients NK cells. On the contrary, KIR2DL2/3 and KIR3DL1 restored promptly, suggesting a sequential expression of KIRs. As interaction between inhibitory KIRs and their ligands are essential for NK cells to become functional ("licensing"

process), we can hypothesize that the weaker expression of KIR2DL1 on C2-C2 NK cells alters the licensing process, rendering the NK cells hypo-responsiveness.

**Conclusion:** Weak capacity of degranulation, low expression of CD16, and recipient C2-C2 is correlated with a worse outcome after RIC-UCBT in a prospective trial for AML patients. These features can reflect an alteration of the NK licensing process and might have impact on clinical outcome after UCBT.

**Disclosure of Interest:** None declared.

#### O145

##### **HLA Loss Leukemia Relapses after Partially-Mismatched Allogeneic HSCT: q Unique Model to Investigate Natural Killer Cell Dynamics**

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**Introduction:** Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) represents the best therapeutic option for many hematological malignancies; nevertheless, relapses remain frequent, requiring further investigations.

Up to one-third of AML relapses after haploidentical HSCT are due to leukemic variants that evade donor T cell control through the de novo genomic loss of the mismatch HLA haplotype.

Still, these "HLA loss" variants should in principle represent excellent targets for alloreactive donor Natural Killer (NK) cells. Aim of this study is to investigate NK cell dynamics in this peculiar context to understand the biological bases of their failure in controlling or preventing the occurrence of HLA loss relapses.

**Material (or patients) and methods:** We analyzed a cohort of 30 patients who experienced HLA loss relapses after haploidentical ( $n=28$ ) or unrelated donor ( $n=2$ ) HSCT. NK cell alloreactivity was predicted according to the Perugia algorithm. KIR typing was performed using a commercially-available kit. The phenotypic features of circulating NK cells were analyzed in 8 patients at the time of HLA loss relapse, in 9 at the time of non-HLA loss "classical" relapse, in 10 long-term remission patients and in 17 healthy individuals, employing a multiparametric flow cytometry panel of 27 markers involved in NK cell target recognition, activation, maturation and exhaustion, and the bHSNE bioinformatic tool for high dimensional single-cell analysis.

**Results:** Based on donor-recipient HLA typing, NK cell alloreactivity was predicted in 18/30 (40%) patients at the time of HSCT and gained in additional 7 (23%) as a consequence of the loss of the mismatched HLA haplotype from AML blasts.

KIR genotyping documented that most of the HSC donors for HLA loss patients carried high numbers of activating KIR genes, and potentially alloreactive single KIR<sup>+</sup> NK cells were detectable in all study groups, including in particular patients with HLA loss relapse.

However, when compared to their counterparts from healthy individuals, NK cells from transplanted patients expressed significantly lower levels of SIGLEC9 ( $P < 0.0001$ ), a receptor shown to be relevant for tumor immunosurveillance. Moreover, comparing HLA loss and classical relapses we detected in HLA loss patients lower frequencies of NK cells expressing the activating receptor NKG2C, possibly a consequence of the lower incidence of CMV reactivations in this subgroup, and higher expression of exhaustion markers, including in particular TIM3 ( $P < 0.01$ ). Accordingly, bHSNE maps demonstrated differential clustering of NK cells between the two groups

of relapses, mainly explained by the aforementioned differences.

**Conclusion:** Even though most of the patients with HLA loss relapses satisfied the conditions for predicted NK alloreactivity, this was not enough to prevent clinical relapse. Defective immunosurveillance was not due to lack of activating KIR genes or of single KIR<sup>+</sup> NK cells, but might rather be explained by relative lack of NKG2C<sup>+</sup> cells and by the upregulation of exhaustion markers. Taken together, our data evidence that the repertoire of circulating NK cells at the time of HLA loss is largely defective. As a solution, we propose that therapeutic protocols employing freshly isolated mature donor NK cells might be tested for the prevention and treatment of HLA loss relapses.

**Disclosure of Interest:** None declared.

#### O146

##### **Screening and Quantitative Monitoring of Calreticulin (CALR) Type-2 Positive Patients with Myelofibrosis Following Allogeneic Stem Cell Transplantation using a novel Digital-PCR Assay**

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**Introduction:** The recent discovery of the somatically acquired calreticulin mutation in about 30% of the myelofibrosis patients provided a new diagnostic marker, which can also be used as marker of minimal residual disease (MRD) following curative treatment with allogeneic stem cell transplantation (ASCT). More than 80% of CALR mutations are of two types: type-1 variants result from a 52-bp deletion and produce the protein change p.L367fs\*46, and type-2 variants are caused by a 5-bp insertion and produce the protein change p.K385fs\*47. Whereas Sanger as well as next-generation sequencing (NGS) readily allow detection of CALR mutations in newly diagnosed patients, their applicability for MRD detection is limited. Real-time quantitative PCR (qPCR) has therefore been suggested as a potential alternative. In order to combine the increased sensitivity of qPCR with the excellent accuracy of digital PCR (dPCR) we developed a duplex dPCR assay detecting the CALR type-2 mutation in combination with its wild-type allele.

**Material (or patients) and methods:** To address sensitivity and reliability of the novel dPCR assay we first tested it on a new, UKE-1 derived cell line harboring one copy of the CALR type-2 mutation (UKE-1CALR2), which was established by lentiviral gene transfer and single-cell sorting. We generated serial dilutions of UKE-1CALR2 in buffy-coat (BC) cells, isolated DNA and submitted it to dPCR. Using 120 ng EcoRI-restricted genomic DNA we detected up to one UKE-1CALR2 in 10,000 BC cells (0.01%) indicating excellent sensitivity. As expected the detection limit could be further increased by applying the p.K385fs\*47-specific dPCR as a singleplex assay.

**Results:** Using our new technique we next performed MRD analysis in CALR+ patients who underwent allogeneic stem cell transplantation and compared results with respective qPCR data. Out of 143 patients with myelofibrosis who underwent allogeneic SCT 92 were JAK2V617 positive, 4 MPL positive and 35 CALR positive. Out of these 35 patients 21 harbored the CALR type-1 and eight the CALR type-2 mutation. In seven out of those eight patients both qPCR and digital PCR could be applied for MRD monitoring after SCT. In 3/7 patient qPCR as well as dPCR were negative on day +20, +100 and +180 after transplantation, respectively. In 2 patients dPCR remained positive at days +100 and +180 days before turning negative, whereas qPCR was already negative. In one patient dPCR remained positive 6 months after SCT, whereas qPCR was negative since day +80. None of the above patients had experienced clinical relapse. In contrast, there was one patient who relapsed 28 months after transplantation. That patient was MRD-negative by qPCR until one month before relapse, whereas dPCR, which initially also became

negative after SCT (day +180), converted to positivity already 1 year after transplantation and was steadily increasing until clinical relapse.

**Conclusion:** Our data indicates that the new CALR type-2-mutation specific dPCR assay combines excellent accuracy with high sensitivity thus allowing the monitoring of deep molecular remission and the early detection of MRD in relapsing patients with myelofibrosis after stem cell transplantation.

**Disclosure of Interest:** None declared.

#### O147

##### **Anti-Interferon gamma monoclonal antibody NI-0501: A new option to render allogeneic HSCT for HLH safer and more effective?**

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**Introduction:** High production of IFN $\gamma$  plays a critical role in the pathophysiology of primary HLH (pHLH), a rare immune regulatory disorder lethal if untreated. In animal studies, high IFN $\gamma$  levels can also impair HSC differentiation and mediate HSCT rejection. Immune-chemotherapy, primarily etoposide-based regimens, is at present the approach used to control HLH and bring patients to curative allogeneic HSCT (allo-HSCT). In spite of recent attempts to optimize treatment regimens, mortality and morbidity remain high, in part due to drug-related toxicities. NI-0501 is a fully human, high affinity, anti-IFN $\gamma$  mAb that binds to and neutralizes human IFN $\gamma$ .

**Material (or patients) and methods:** An open-label Phase 2 study is conducted in Europe and US to evaluate NI-0501 treatment in pHLH children. NI-0501 was administered at initial dose of 1 mg/kg every 3 days, with possible dose increase guided by PK and clinical response on initial background dexamethasone (dexa) of 5-10 mg/m<sup>2</sup>. Treatment duration ranged from 4 to 8 weeks. 16 patients were enrolled: 8F/8M, median age 1.2yr (range 0.2-13); 14 patients received NI-0501 as 2<sup>nd</sup> line treatment after having failed conventional therapy or being intolerant to it. Two patients have been treated with NI-0501 in 1<sup>st</sup> line; 12 patients carried a known HLH genetic defect (4 FHL2, 2 FHL3, 1 FHL4, 3 GS-2, 1 XLP1, 1 XLP2). Most patients were at the severe end of HLH spectrum and carrying significant toxicities from previous HLH treatments. At baseline, CNS involvement was recorded in 4 patients. One patient was excluded from efficacy analysis due to a subsequent secondary HLH diagnosis.

**Results:** Of the 15 evaluable patients, 13 have completed treatment and 2 are ongoing. NI-0501 treatment significantly improved parameters of HLH disease activity. Nine of 13 patients achieved a satisfactory response: 7 patients proceeded to allo-HSCT, and transplant is planned for 2 patients. Four patients had insufficient response to NI-0501: 2 patients were also given allo-HSCT, 2 died prior to HSCT of HLH/multi-organ failure. CNS signs and symptoms resolved in the evaluable patients. Significant ( $\geq 50\%$ ) tapering of dexa dose was possible in 10/13 patients (median dose: 10 mg/m<sup>2</sup> at baseline vs 4 mg/m<sup>2</sup> at end of treatment;  $P=0.023$ ). In all responders, IFN $\gamma$  neutralization at time of HSCT was demonstrated by undetectable levels of CXCL9, a chemokine exquisitely IFN $\gamma$ -induced. The donor was an HLA-identical sibling in 1 patient, a haplo-identical relative in 1 and an unrelated donor in the others (including cord blood cells in 1 case). Two cases of acute GvHD occurred: 1 resolved and 1 led to death together with bacterial sepsis. The remaining

8 patients are alive and disease-free, the median follow-up being 2.6 mo (range 0.1-2 yr). No infection known to be favored by IFN $\gamma$  neutralization was reported.

**Conclusion:** Neutralization of IFN $\gamma$  by NI-0501 offers an innovative targeted and effective approach to HLH management before allo-HSCT. Furthermore, NI-0501 can spare short- or long-term toxicities reported for etoposide-based regimens, thus translating into a reduced risk of allo-HSCT-related complications. Preliminary data on exclusive IFN $\gamma$  blockade in an HSCT mouse model (Novimmune unpublished data) show improved donor BM chimerism, strengthening the potential additional benefit of IFN $\gamma$  neutralization in HLH. Confirmation of these latter findings is expected from ongoing studies.

**Disclosure of Interest:** F. Locatelli: None declared, M. Jordan Conflict with: Novimmune Consultancy, C. Allen Conflict with: Novimmune Consultancy, S. Cesaro: None declared, F. Fagioli: None declared, M. Henry: None declared, C. Rizzari: None declared, C. Rossig: None declared, J. Sevilla: None declared, M. Ballabio Employee of: Novimmune sa, W. Ferlin Employee of: Novimmune sa, C. De Min Employee of: Novimmune sa.

#### O148

##### **Impact of drug development on the use of Stem Cell Transplantation A report by the activity survey of the European Society for Blood and Marrow Transplantation (EBMT)**

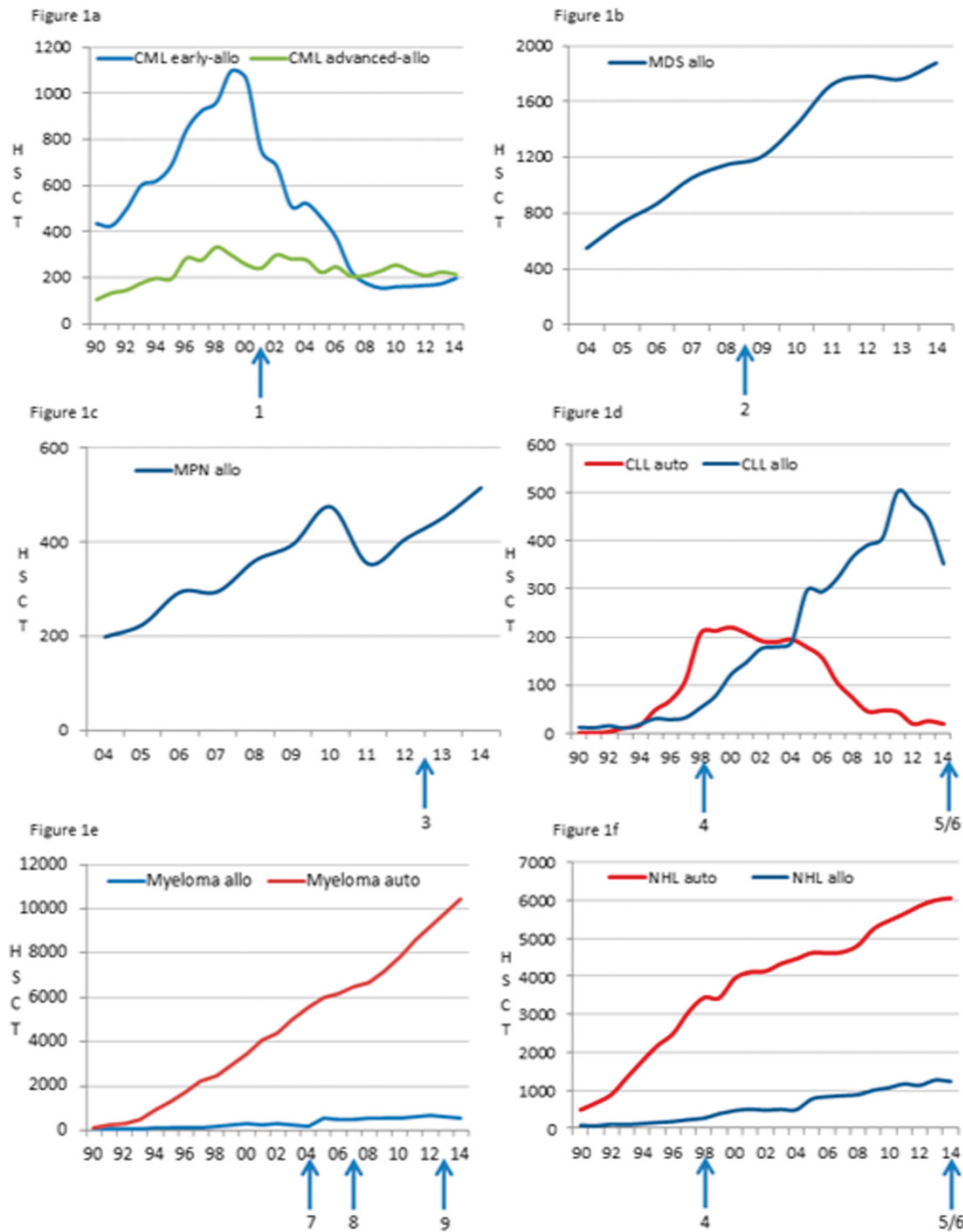
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**Introduction:** HSCT is used with increasing frequency in Europe, in 2014 for the first time over 40'000 transplants were reported. Transplant related mortality remains high between 10-20% in allogeneic HSCT and around 1% for autologous HSCT, high dose chemotherapy is toxic and demanding for patients. Progress has been made over the years but toxicity HSCT is still a challenge. Drug development is accelerating and many new drugs have been developed and marketed for hematologic malignancies in the past few years. Some of the more targeted drugs have limited toxicity and it is of interest to examine whether these have changed the use of HSCT for selected indications. A specific effective drug may replace HSCT or lead to decreased use whereas another drug may enhance HSCT use and function as a "bridge to transplant".

**Material (or patients) and methods:** The EBMT activity survey collects data on indications for HSCT in Europe. We analyzed activity in a number of diseases with advances in drug development. Here we show data on HSCT for CML, MDS, MPN, CLL, Myeloma and NHL. Numbers of HSCT over the years for specific indications are plotted; arrows indicate the date of EMA marketing authorization.

**Results:** Tyrosine kinase inhibitors dramatically changed the allogeneic HSCTs done for early but not for advanced CML (Fig. 1a). Fig.1b shows MDS allogeneic HSCT where marketing of azacitidine may have increased transplant activity, similarly in MPN (Fig. 1c) there appears to be no or only a limited drop



Legend: Effect of drug development on HSCT in selected indications; 1a: CML early and advanced allogeneic HSCT; 1b - MDS allogeneic HSCT; 1c - MPN allogeneic HSCT, 1d - CLL allogeneic and autologous HSCT, 1e - HD allogeneic and autologous HSCT and 1f - myeloma allogeneic and autologous HSCT  
 Drugs: 1 imatinib, 2 azacitidine, 3 ruxolitinib, 4 rituximab, 5 ibrutinib, 6 idelalisib, 7 bortezomib, 8 lenalidomide, 9 pomalidomide

after introduction of ruxolitinib. For CLL (Fig. 1d) HSCT is shown along with marketing authorization for Rituximab and Ibrutinib/Idelalisib. Autologous HSCT for CLL has decreased since 2004 after publication of trials showing PFS but no OS advantage, and it appears that allogeneic HSCT rates are dropping after introduction of Ibrutinib / Idelalisib. Whether these are a "game changer" as was Imatinib for CML requires additional follow-up. Fig 1e shows HSCT for Myeloma where development of proteasome inhibitors and new IMiDs appears not to impact transplant rates and similarly in NHL (Fig 1f) no impact of drug development is apparent.

**Conclusion:** New drugs may have different effects on the use of autologous or allogeneic HSCT; highly effective drugs may replace HSCT whereas other drugs may be used to improve the patient's condition to allow for HSCT.  
**Disclosure of Interest:** None declared.

O149

**Mesenchymal Stem Cell: Does it Work in an Experimental Model with Acute Respiratory Distress Syndrome?**

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**Introduction:** We hypothesized that bone marrow-derived mesenchymal stem cells (BM-MSCs) would have a possible role in the treatment of acute respiratory distress syndrome (ARDS).

**Material (or patients) and methods:** ARDS disease model was developed in Wistar albino male rats by intratracheal instillation of physiological saline solution. Anesthetized and tracheotomized rats (n08) with ARDS were pressure-controlled ventilated. Isolated and characterized rat (r-) BM-MSCs were labeled with GFP gene, and introduced in the lungs of the ARDS ratmodel. After applying of MSCs, the life span of each rat was recorded. When rats died, their lung tissues were removed for histopathological examination. Also the tissue sections were analyzed for GFP labeled rBM-MSCs and stained for vimentin, CK19, proinflammatory (MPO, IL-1β, IL-6 and MIP-2) and anti-inflammatory [IL-1ra and prostaglandin E2 receptor (EP3)] cytokines.

**Results:** The histopathological signs of rat-model ARDS were similar to the acute phase of ARDS in humans. rBM-MSCs were observed to home in lung paranchyma. Although the infiltration of neutrophils slightly decreased in the interalveolar, peribronchial and perivascular area, a notable improvement was determined in the degree of hemorrhage, edema and hyaline membrane formation in rats treated with rBM-MSCs. Also decreased proinflammatory cytokines levels and increased the intensity of anti-inflammatory cytokines were established. Therefore MSCs could promote alveolar epithelial repair by mediating of cytokines from a proinflammatory to an antiinflammatory response (Figure 1).

**Conclusion:** As a novel therapeutic approach, mesenchymal stem cell treatment with intratracheal injection could be helpful in the management of critically ill patients with ARDS.

**Disclosure of Interest:** None declared.

O150

**IL-33/ST2 Triggering of IL9 Secreting T Cells Alters the Balance of Fatal Immunity and Tumor Immunity**

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**Introduction:** As one of the most validated immunotherapies to date, allogeneic hematopoietic cell transplantation is a potentially curative option for high-risk hematological malignancies, particularly chemo-resistant acute myeloid leukemia (AML). However, graft-vs-leukemia (GVL) activity is often accompanied with high risk of allo-antigen reaction of donor T cells against normal host tissue, inducing aGVHD. We have shown that an elevated plasma level of soluble (s)ST2 in HCT patients is a risk factor for severe GVHD<sup>1</sup>. ST2 blockade reduces sST2-producing T cells while maintaining protective membrane (m)ST2-expressing T cells during aGVHD<sup>2</sup>. A novel IL-9 producing T helper subset, Th9, expresses mST2<sup>3</sup>. Furthermore, Th9 cells and IL-9 producing CD8 cytotoxic (Tc9) cells have higher antitumor activity than Th1 and Tc1 cells in

melanoma models<sup>4,5</sup>. Our hypothesis is that ST2/IL-33 activation in Th9 and Tc9 (together T9) cells alleviates GVHD and increases GVL activity.

**Material (or patients) and methods:** Allogeneic donor B6 or syngeneic Balb/C T9 cells were generated from splenic T cells cultured with IL-4 + TGFβ or IL-4 + TGFβ+ IL-33 (T9<sub>IL-33</sub>). Concurrently, T0 (no cytokines), T1 (with IL-12), and T2 (with IL-4) cell subsets. These cell types were individually adoptively transferred with bone marrow cells into allogeneic irradiated major MHC-mismatched recipients to induce GVHD. For GVL studies recipient mice also received syngeneic retrovirally transduced MLL-AF9 leukemic cells.

**Results:** We found that T9<sub>IL-33</sub> cells express higher mST2 and PU.1, the master transcription factor of Th9<sup>6</sup>, compared to all other subsets including T9. Adoptive transfer of T9<sub>IL-33</sub> cells with bone marrow cells in a murine model of HCT resulted in less severe GVHD compared to transfer of T0, T1, T2, T9, and T9<sub>IL-33</sub> cells generated from ST2<sup>-/-</sup> or IL-9<sup>-/-</sup> T cells. Furthermore, *in vivo* GVL experiments with A20 lymphoma cells or MLL-AF9 transduced leukemia and T9<sub>IL-33</sub> cells resulted in increased survival compared to transfer of WT, T1, T9 or T9<sub>IL-33</sub> cells generated from ST2<sup>-/-</sup> or IL-9<sup>-/-</sup> T cells. Transcriptome analysis of WT T9<sub>IL-33</sub> versus ST2<sup>-/-</sup> T9<sub>IL-33</sub> showed higher expression of cytolytic molecules such as Granzyme A, KLRK1, CD160, and Granzyme B in both CD4 and CD8 sorted WT T9<sub>IL-33</sub> cells. T9<sub>IL-33</sub> cells also demonstrated higher *in vitro* anti-leukemic activity when incubated with MLL-AF9 leukemia as compared to ST2<sup>-/-</sup> T9<sub>IL-33</sub> cells. Human T9 cells are poorly explored; we demonstrated that differentiation of human T9 cells in the presence of IL-33 enhanced granzyme B and IL-9 production. Human T9<sub>IL-33</sub> also demonstrated higher anti-leukemic cytolytic activity when incubated with MOLM14, an aggressive tumor cell line with FLT3/ITD mutations, as compared to T9 cells. Furthermore, investigations into the possible mechanism of activation revealed upregulation of CD8α in transcriptome and protein levels. We hypothesized that CD8α might be the contact-dependent component. CD8α blockade with neutralizing antibody during human T9<sub>IL-33</sub> differentiation reduced the cytotoxicity of both murine T9<sub>IL-33</sub> and human T9<sub>IL-33</sub> cells.

**Conclusion:** Our observations suggest that adoptive transfer of T9<sub>IL-33</sub> cells represents a promising cellular therapy following HCT.

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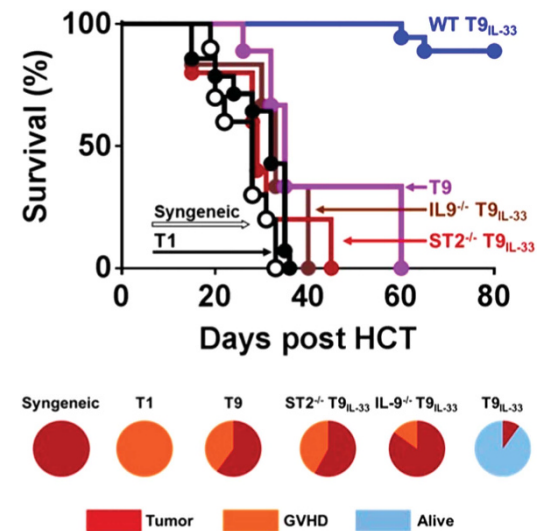
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**Disclosure of Interest:** None declared.





O151

**Stem Cell Transplantation with Fludarabine and Melphalan Conditioning for Children with Acquired Bone Marrow Failure: A Nationwide Retrospective Study**

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**Introduction:** Stem cell transplantation (SCT) for acquired bone marrow failure (aBMF) has been established treatment, and the optimal conditioning regimens according to various settings have been developed. Fludarabine (FLU)/cyclophosphamide (CY)-based regimen is one of the standard conditionings for children with aBMF. However, we recently found that the regimen was relevant to the increase of risk for poor hematological recovery or bone marrow aplasia even with full donor chimerism after SCT (donor-type aplasia). The purpose of this study is to evaluate whether the use of FLU/melphalan (MEL)-based conditioning instead of the FLU/CY-based regimen can reduce the risk for donor-type aplasia.

**Material (or patients) and methods:** We retrospectively reviewed the clinical data of 603 patients (< 16 years) with aBMF (aplastic anemia and refractory cytopenia of childhood) who received the first SCT from 2000 to 2013 and registered in the Japan Society for Hematopoietic Cell Transplantation Registry. Totally, 49 patients received the FLU/MEL-based regimen. Of the 49, 21 patients received SCT from a related donor, whereas 28 received it from an unrelated donor. The stem cell source was bone marrow in 36 patients, peripheral blood in 1, or cord blood in 12. The conditioning regimen was based on FLU (100-180 mg/m<sup>2</sup>) and MEL (70-180 mg/m<sup>2</sup>), ATG was included in the regimen in 29 patients, while low dose irradiation was used in 38 patients.

**Results:** The 5-year overall survival and event free survival (EFS) of patients who received the FLU/MEL-based regimen was 91%. Engraftment was achieved in 98% of patients and secondary graft failure including donor-type aplasia was not observed. Regarding the MEL dose, the favorable survival in patients who received 120 mg/m<sup>2</sup> or more (140 mg/m<sup>2</sup> was the most commonly used) was observed (100% vs. 62%; *P* = 0.006). In the setting of cord blood transplantation (CBT), all 9 patients who did not receive ATG as conditioning regimen engrafted and survived, whereas 2 of 3 patients who received ATG died. Notably, all patients who received bone marrow transplantation (BMT) were alive without any complication. We then compared the outcomes in the setting of BMT with the FLU/MEL-based regimen (*n* = 36) to those with the standard FLU/CY-based regimen (*n* = 270). The EFS was inferior in patients treated with the FLU/CY-based regimen, although this difference was not statistically significant (86% vs. 100%; *P* = 0.07). With the FLU/CY-based regimen, engraftment was achieved in 98% of patients, whereas secondary graft failure including donor-type aplasia was seen in 21 patients.

**Conclusion:** The FLU/MEL-based regimen can be a new standard conditioning regimen for children with aBMF especially in the setting of BMT. CBT using the FLU/MEL-based regimen without ATG also provided excellent outcomes. Given this, a prospective study on SCT with this conditioning for aBMF children is now planned by the Japan Childhood Aplastic Anemia Study Group.

**Disclosure of Interest:** None declared.

O152

**Excellent outcome of Hematopoietic stem cell transplantation in children with acquired aplastic anemia from alternative donor**

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**Introduction:** Immunosuppressive therapy (IST) is applied to children with aplastic anemia (AA) as first line therapy when a HLA matched family donor is not available. Response to IST can be anticipated in 50-60% of patients, relapse in about 20% of responders and clonal evolution to MDS in 10% of long term survivors. We recently reported that the absence of a minor paroxysmal nocturnal hemoglobinuria population and a short telomere length is an efficient predictor of poor IST response at 6 months (19% in poor prognosis group vs 70% in others) in children with acquired AA (Narita, A et al. Haematologica 2015). This finding let us reevaluate recent outcome of hematopoietic stem cell transplantation (HSCT) in children with AA to address a question who should be treated by up-front HSCT rather than IST for better decision-making treatment algorithms.

**Material (or patients) and methods:** We analyzed 67 children with AA who underwent HSCT in Nagoya university hospital between Jan. 2000 and Mar. 2015. Forty patients received HSCT from HLA matched bone marrow donor (23 family donors and 17 unrelated donors). Twenty seven patients received HSCT from HLA mismatched donors (19 mismatched unrelated BM donors, 7 HLA haploidentical family donors and 1 unrelated cord blood). Twenty three patients transplanted from HLA matched family donor received cyclosporine and short term MTX as GVHD prophylaxis and all other patients received tacrolimus and short term MTX. For haplo-HSCT, patients received a conditioning regimen consisting of fludarabine 30 mg/m<sup>2</sup> i.v. x 4 days, melphalan 70 mg/m<sup>2</sup> i.v. x 2 day and TBI 4-5Gy in two fractions. BMT was conducted on day0 and G-CSF mobilized peripheral blood stem cells (PBSCT) were infused on day6 to boost numbers of stem cells in haplo-HSCT while ATG were given at 10mg/kg prior to BMT and at 5mg/kg one day before PBSCT. The conditioning regimen for allogeneic cord transplantation was the same as for haplo-HSCT but without ATG.

**Results:** The median age was 9 (range; 1 to19) years old in this cohort. The engraftment was achieved in 65 out of 67 (97.0%) and the 2 rejected patients from UR-HSCT were rescued by the second urgent haplo-HSCT. All patients from alternative donors achieved engraftment. Out of 40 HLA matched HSCT, acute GVHD grade II or more and chronic GVHD were seen in 2 and 2 respectively. Out of 27 HLA mismatched HSCT, acute GVHD grade II or more and chronic GVHD were seen in 6 and 7 respectively. EBV reactivations were observed in 8 and those patients were treated by rituximab. Only one patient developed rituximab resistant CD20 negative EBV associated lymphoproliferative disease and was rescued by EBV specific CTLs therapy. Five years overall survival was 97.0% (95%CI, 88.6-99.2%). All 27 patients transplanted from HLA mismatched donors are alive. Cause of 2 deaths (1 HLA matched sibling donor, 1 HLA matched UR donor) was pneumonia. Sixty five out of sixty seven patients are alive with the median follow-up period of 98 months (range: 9 months to 145 month).

**Conclusion:** Excellent outcome of HSCT were observed in children with AA from three alternative donor options. These results combined with a previously reported efficient predictor of poor IST response could give us rationale to conduct a prospective clinical study of up-front HSCT from alternative donors in carefully selected patients.

**Disclosure of interest:** None declared.

#### O153

##### **Eculizumab followed by allogeneic hematopoietic stem cell transplantation (HSCT) for hemolytic paroxysmal nocturnal hemoglobinuria / severe aplastic anemia (hPNH/SAA)**

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**Introduction:** Allogeneic HSCT is a standard curative approach for SAA, but concomitant hPNH may increase the risk of post-transplant complications. Recently published small series and individual cases suggest the effectiveness of pre-HSCT anti-complement therapy with eculizumab in patients with both hPNH and severe bone marrow failure (Kulasekararaj A.G. et al., 2013; Maschan M. et al., 2013).

**Material (or patients) and methods:** Eight patients (male 3, female 5; median age 24 years, range 14-30) with transfusion-dependent hPNH/SAA receiving HSCT with prior eculizumab therapy were prospectively evaluated between April 2014 and December 2015. Median time from SAA diagnosis to HSCT was 68 months (range, 6-144). All patients had evidence of intravascular hemolysis with median LDH level of 3.5 ULN (range, 1.5-7.9) before eculizumab therapy. Pre-HSCT median PNH clone size was 84% (range, 37-98) and 17% (range, 2-62) in granulocytes and RBC respectively. Conditioning regimens were Flu/Bu8/ATG (*n*=7) and Flu/Cy/ATG (*n*=1). All patients except one were grafted with bone marrow from HLA-Id sibling (*n*=5) and HLA-matched (10/10, *n*=2) or 9/10 (*n*=1) unrelated donor. GVHD prophylaxis consisted of CsA+MTX +/-MMF (*n*=4) and Tacro+MTX+/-MMF (*n*=4). Two patients were on chronic eculizumab treatment for 1.4 and 3.2 years, while the remaining 6 patients received a short course (2-15 weeks) immediately before to HSCT.

**Results:** Eculizumab therapy resulted in resolution of hemolysis in all patients with the median LDH level at Day 0 of 0.86 ULN (range, 0.58-1.42). No new thrombotic events and VOD signs occurred during eculizumab treatment and after HSCT. Engraftment was observed in 7 patients with median time to ANC > 500 of 25 days (range, 14-30). One patient died of sepsis on D+23 before hematopoietic recovery. Acute GVHD grade 1 and limited skin chronic GVHD were observed in one case each. Minor PNH clone (median 0.08%, range 0.02-0.56, in granulocytes) was still detectable by high sensitive flow cytometry at the time of engraftment and disappeared completely in all cases by Day +100. With a median follow-up of 8 months (range, 1-14) for surviving patients, seven patients are alive with sustained engraftment, full donor chimerism and PNH-free. The estimated 1-year probability of both overall and failure-free survival was 87.5% (95% CI, 65-100%).

**Conclusion:** Eculizumab effectively inhibits intravascular hemolysis in hPNH/SAA patients before HSCT. Reduced intensity Bu8/FLU/ATG conditioning provides an acceptable engraftment rates in heavily transfused hPNH/SAA patients. Eculizumab followed by allogeneic HSCT seems to be a promising curative option for hPNH/SAA and warrants further investigation in a large prospective cohort with longer follow-up.

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V. Ovetchkina: None declared, Y. Gudozhnikova: None declared, Y. Zalyalov: None declared, O. Slesarchuk: None declared, E. Babenko: None declared, M. Estrina: None declared, A. Alyanskiy: None declared, S. Bondarenko: None declared, B. Afanasyev: None declared.

#### O154

##### **Unusual Association of Aplastic Anemia and Lymphoid Neoplasm: A Survey of the Severe Aplastic Anemia Working Party of the EBMT**

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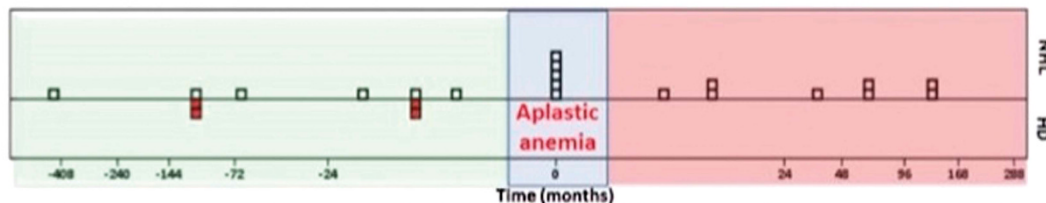
**Introduction:** The association between aplastic anemia (AA) and lymphoid neoplasms is unusual. We do not yet know whether there is causality or simply coincidence in this association. Data on frequency, diagnosis and management of patients (p) with such association are lacking. The SAAWP of the EBMT aimed to collect data on cases with AA and a lymphoid neoplasm.

**Material (or patients) and methods:** First, a search for this association was performed within the registry of the EBMT. In a second step, centers reporting cases were asked for details on time of occurrence, type of therapy and outcome for each disease. The EBMT registry contacted 294 reporting centers, and 83 (28%) replied.

**Results:** In total 23 cases with AA and lymphoma were reported by 12 centers from 7 countries. There were 15 males (65%), median age at 1<sup>st</sup> diagnosis was 48 years(y) (range 10-74). First diagnosis was AA in 8/23 (34%) cases, and lymphoma in 10 (44%) cases. In 5 (22%) cases both diagnosis were performed at the same time. The diagnosis of acquired AA was performed between 1983-2013, median age at AA diagnosis was 57y (10-74), 16/21p (66%) showed severe or very severe AA, 5/21p (24%) moderate AA; 2 cases were of unknown severity. Only 1 of 19p presented a PNH clone, and 1 of 19p had a cytogenetic anomaly. In 3/19 (15%) a viral association was demonstrated. The most frequent treatment administered for AA was a regimen including ATG (14p) or Cyclosporin alone (6p). Remission status at last control: complete remission (CR) 11p(48%); partial remission (PR) 4p (17%), refractory 4p (17%), progression to MDS 1p (4%), unknown 3p (13%). The diagnosis of lymphoid neoplasm was performed between 1958-2012, median age at diagnosis was 49y (12-74). Four p (17%) had a Hodgkin lymphoma, 19/23 p (83%) had non-Hodgkin lymphoma, all of them B-cell lymphoid neoplasms: diffuse large B cell lymphoma (4); follicular lymphoma (3); lymphoplasmacytic lymphoma (2); nodal marginal zone B cell lymphoma (2), CLL (2), mantle cell lymphoma; splenic marginal zone lymphoma and multiple myeloma 1 case each, other or unspecified lymphoma (3). The lymphoma was treated in 95% of cases, 48% required more

[0154]

Time of appearance of lymphoma in relation to aplastic anemia (time 0); NHL, non Hodgkin lymphoma (empty box); HD, Hodgkin disease (solid-filled box)



**Lymphoma first (n=10)**  
 Treatment AA  
 - ATG regimen 4/10  
 - CSA alone 4/10  
 - Growth factors 1/10  
 - Allo HSCT 1/10  
 Type of lymphoma  
 - B-cell lymphoma 6/10  
 - Hodgkin lymphoma 4/10  
 Treatment Lymphoma  
 - Chemotherapy 6/10  
 - Alemtuzumab 1/10  
 - Other  
 HSCT 5/10  
 Reasons for HSCT  
 - AA 5/5  
 Alive 5/10  
 Causes of death  
 - HSCT related 2/5  
 - AA related 3/5

**AA and lymphoma (n=5)**  
 Treatment AA  
 - ATG regimen 2/5  
 - CSA alone 2/5  
 - Alemtuzumab 1/5  
 Type of lymphoma  
 - B-cell lymphoma 5/5  
 - Hodgkin lymphoma 0/5  
 Treatment Lymphoma  
 - Chemotherapy 3/5  
 - Monoclonal AB alone 2/5  
 - Other 0  
 HSCT 2/5  
 Reason for HSCT  
 - AA 2/2  
 Alive 3/5  
 Causes of death  
 - AA related 2/2

**Aplastic anemia first (n=8)**  
 Treatment AA  
 - ATG regimen 8/8  
 - CSA alone 0/8  
 - Alemtuzumab 0/8  
 Type of lymphoma  
 - B-cell lymphoma 8/8  
 - Hodgkin lymphoma 0/8  
 Treatment Lymphoma  
 - Chemotherapy 4/8  
 - Monoclonal AB alone 2/8  
 - Other/none 2/8  
 HSCT 3/8  
 Reason for HSCT  
 - AA 2/3  
 - Lymphoma 1/3 (?)  
 Alive 6/8  
 Causes of death  
 - HSCT related 2/2

than 1 line therapy. Ten of the 23 p received eventually allo HSCT, all but one for the treatment of AA. At last follow-up, 14/23p (61%) were alive, and 9 had died: the cause of death was transplant related mortality (4) or AA related mortality (5). None of the deaths were due to the lymphoma. The relationship between AA and lymphoma is shown in the figure, where time of appearance of the lymphoma (represented as a box) is shown in relation to AA (considered as time 0), and the three clinical presentations (lymphoma first, AA and lymphoma together, AA first) are outlined.

**Conclusion:** We confirm that the rare association of AA and lymphoma may occur in any of the three described clinical presentations. Patients were clinically very heterogeneous. Sequential presentations were more frequent and concomitant presentation did not show a particular profile. The management was heterogeneous but the outcome was mainly affected by the AA, not the lymphoma. This is the largest series with this rare association evaluated so far; while there is no evidence of a common pathophysiologic mechanism we can hypothesized that different underlying mechanisms may be involved in the different forms of presentation. Causality remains however controversial.

**Disclosure of Interest:** None declared.

**O155 Haematopoietic stem cell transplantation in  $\beta$ -thalassaemia and sickle cell disease: The Spanish experience**

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**Introduction:** Major changes in the prevalence of  $\beta$ -thalassaemia and sickle cell disease (SCD) have taken place in Spain in the last few years because of the immigration process that started at the end of the 20<sup>th</sup> century. This increase in the prevalence of hemoglobinopathies is raising the need for improved prevention and treatment services.

Here, we briefly review the current situation in Spain regarding neonatal screening and treatment guidelines and we present a retrospective analysis of the Spanish results in hematopoietic stem cell transplantation in  $\beta$ -thalassaemia and sickle cell disease (SCD).

**Material (or patients) and methods:** Data from GETMON (Spanish Group for Bone Marrow Transplantation) was

analysed. The Spanish paediatric HSCT units performing HSCT in  $\beta$ -thalassaemia or sickle cell disease were identified and were sent a questionnaire and seven of eight HSCT units contacted responded.

The analysed data included patient characteristics, disease characteristics, HSCT procedure and HSCT outcome.

**Results:** Data from 43 patients diagnosed with  $\beta$ -thalassaemia and 22 patients with SCD was analysed.

Indication for transplant was transfusion dependency for  $\beta$ -thalassaemia and recurring or severe symptoms for SCD patients. At transplant,  $\beta$ -thalassaemia patients were slightly younger (6.3 years vs 8.6 years) and had been more heavily transfused than SCD patients.

Conditioning regimen was myeloablative and based on busulfan for most patients. GVHD prophylaxis was a combination of cyclosporine plus MTX or methylprednisolone or MMF for most patients but varied among centres and also depended on patient characteristics and risk of toxicity.

All grafts had an adequate cell dose. 1 patient experienced primary graft failure and 5 secondary graft failure. Quimerism analysis demonstrated full donor chimerism in 56 patients (86%) and stable mixed chimerism in 3 patients (4.69%).

VOD was diagnosed in 5 patients and completely resolved and neurological toxicity in the form of PRES in 14 patients. Incidence of acute and chronic GVHD were limited; 4 patients experienced extensive chronic GVHD.

OS was 92% for  $\beta$ -thal and 85% for SCD patients. Although not statistically significant, there seems to be an improvement over time and patients transplanted after 2010 seem to have a better outcome. No differences in outcome could be detected when analysing other patient or transplant procedure variables.

	$\beta$ -Th $\beta$ -Thalassaemia (43 patients) alassaemia (43 patients)	SCD (22 patients) SCC
<i>Donor</i>		
MSD	29	21
MFD	5 (1: 11/12)	0
MUD	8	
MMUD	1	1 (4/6 UCB)
<i>Source of stem cells</i>		
Bone marrow	35	19
Peripheral blood	3	0
Cord Blood	1	2
CB+Bone marrow	4	1
<i>Serotherapy</i>		
No	29	2
ATG	13	8
Campath	1	12

**Conclusion:** The increase in Hb gene prevalence in Spain is raising the need for improved prevention and treatment services of these patients. Our retrospective analysis of the Spanish results of HSCT in beta-thalassaemia and SCD shows encouraging results so far. However, it should be seen as the starting point for continuous improvement of the outcome, both in survival rates and in reduction of toxicity.

**Disclosure of Interest:** None declared.

## O156

### Awareness about Hematopoietic Stem Cell Transplant as Curative Option for Sickle Cell Disease among Physicians: Survey from Sickle Dominated Area in Africa

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**Introduction:** Sickle cell disease (SCD) is associated with huge financial & medical burden. Supportive treatment has remained standard of care for management of SCD in past but it comes with huge financial burden over a period of time. Even with best possible supportive care, the quality of life is still very poor and the life expectancy is rarely beyond 50 years. In recent years hematopoietic stem cell transplant (HSCT) has emerged as a potential cure for SCD. The overall awareness however about this as a curative option is less among the physicians. We present a survey done in sickle dominated area in Africa.

**Material (or patients) and methods:** A ten point questionnaire was prepared and was sent over to physicians and pediatricians catering SCD via email. Data collected was then analyzed.

**Results:** Questionnaire was given to 60 physicians of whom 55 responded. Sixty four percent physicians were seeing around 11-20 patients per month whereas 36% were seeing around 20-50 cases. Cent percent said that average expenses in managing a sickle patient per year is 2000-5000 USD. Almost 100% parents were asking for option of permanent cure but only 73% physicians were suggesting HSCT as a curative option with guarded prognosis. While counseling about HSCT 27% said major barrier was unawareness among the parents whereas 73% said it was lack of funds which was major deterrent while considering BMT. When asked about success of matched sibling HSCT 37% said it's around 50%, 37% said approx 70% whereas 26% said it's around 90%. When asked the same question about Haploidentical HSCT 55% said its approx 60-70% whereas 18% said it's around 80-90%. Almost 100% said considering risk and benefit, they will advice HSCT for their patients from now onwards. Around 82% agreed to the fact that they will choose transplant physician before transplant center while considering HSCT for their patients.

**Conclusion:** Hematopoietic stem cell transplant can benefit selected subset of SCD. Parents do want to explore option of permanent cure from disease but lack of awareness about the procedure among physicians act as a barrier. From care givers perspective lack of funds was the main deterrent while considering HSCT. More awareness drives need to be done among physicians and care givers regarding HSCT for SCD.

**Disclosure of Interest:** None declared.

## O157

### Transient elastography changes in the liver of major thalassaemia patients after hematopoietic stem cell transplantation

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**Introduction:** Hepatic fibrosis, as a consequence of multiple blood transfusions, may still progress despite successful HSCT in major thalassaemia (MT) patients. Although liver biopsy remains the gold standard for evaluating hepatic fibrosis, recently noninvasive imaging techniques such as transient elastography have emerged. The purpose of this study is to examine the alterations of transient elastography value for evaluation of liver fibrosis in pediatric patients with MT after HSCT.

**Material (or patients) and methods:** Seventy five (43boys, 32girls) pediatric MT patients who were eligible for HSCT enrolled in this study. The median age was 7.5 years (range: 2–14). Liver stiffness was assessed for all patients, before transplantation using both TE, measured in kilopascals (kPa) and liver biopsy based on the Metavir score to determine fibrosis stage based on the Lucarelli classification, and one year after transplantation by TE merely. Simultaneously Hepatic T2\* MRI value and serum ferritin level were measured. **Results:** The median TE score was 4.584 kPa and 5.056 kPa in pre-transplant and ex-thalassemic patients respectively. TE score significantly increased after HSCT ( $P$ -value  $< 0.05$ ), showing the liver status has been worsened. Correlation between TE score and Hepatic T2\* Value was significant ( $r = -0.243$ ,  $P$ -value  $< 0.05$ ). There was no significant difference between hepatic graft-versus-host disease (GvHD) and non-GvHD patients in TE values ( $P$ -value = 0.60).

**Conclusion:** The results of our study demonstrated that hepatic fibrosis in ex-thalassemic patients assessed by TE, progressed after HSCT. Correlation between TE score and Hepatic T2\* Value showed that liver iron overload due to multiple blood transfusions before engraftment can cause deterioration of liver fibrosis. However, large case-control studies accompanied by liver biopsy results after HSCT is recommended.

**Disclosure of Interest:** None declared.

#### O158

##### **Acceptability of the Fertility Preservation Program and Evaluation of the Gonadal Function in Children after Bone Marrow Transplantation**

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**Introduction:** Infertility is a major long term side effect after hematopoietic stem cell transplantation (HSCT). A myeloablative conditioning regimen is associated with high rates of gonadal failure. Several options for fertility preservation are currently available for children i.e. sperm cryopreservation, gonadal tissue cryopreservation but some techniques are still experimental.

**AIMS:** To analyse the acceptability of a fertility preservation program in pediatric HSCT and to evaluate the ovarian function several years after HSCT and to determine which patients are at risk to develop premature ovarian failure.

**Material (or patients) and methods:** All children treated by HSCT since the initiation of the fertility program (1998 for girls and 2009 for boys) in our center were included in the study. Clinical and biological data were collected until the 30 April 2015 and were retrospectively analysed. Evaluation of ovarian function was made for girls older than 13 years. Gonadal function was determined from review of clinical and biological data.

**Results:** From May 1998 to December 2014, 445 children (187 boys, 258 girls) received a first HSCT at a median age of 7.4 years (range: 0.4–18.8). 70% patients had been treated for a malignant pathology and 30% for a non-malignant disease. The conditioning regimen was administered according to the pathology and has been classified as myeloablative conditioning regimen (MAC) with or without total body irradiation (TBI) and reduced intensity conditioning (RIC).

A fertility preservation was proposed by haematologist to 237 children (53%). Twenty five families declined the proposal. 208 families met the fertility specialist and after counselling, 28 (13%) refused the procedure and 23 (11%) presented with a medical contra indication to the surgery. Finally, 161 children had a cryopreservation: 4 postpubertal boys had a sperm cryopreservation, 64 boys underwent testicular biopsy and 93 girls an unilateral ovariectomy at a median age of 8.8 years (0.8–16) and 7.2 (0.6–16.5) for boys and girls, respectively. No side effects of the surgery were reported.

Assessment of ovarian function was available for 69 post pubertal girls with the median time of follow up after HSCT of 5.2 years (1–14, 7). The median age at HSCT for these 69 girls was 10.4 years (1.2–17.9). 62% were treated for a malignant disease and 38% for a non-malignant disease. Conditioning regimen was MAC for 56 girls (81%) with TBI for 18 (26%), and RIC for 13 girls (17%). 28/69 (40%) underwent ovarian cryopreservation before HSCT.

42 girls had developed premature ovarian insufficiency at a median age of 15.3 years (13–19, 5). From them, 11 (26%) were older than 13y at time of HSCT, 38 (90%) received MAC and 26 (62%) had an ovarian cryopreservation.

In multivariate analysis, both HSCT after 13 years and ovarian cryopreservation appeared as significant negative impact factors for ovarian failure ( $P = 0.002$  and  $P = 0.001$ , respectively).

**Conclusion:** Fertility preservation is a major concern for both physicians and patients. Gonadal tissue cryopreservation is the only available technique at time. This technique is well accepted by patients and families. However, in girls this procedure may impair fertility by itself. Pediatric transplanters and fertility physicians have to conduct prospective programs to develop new techniques and increase results.

**Disclosure of Interest:** None declared.

#### O159

##### **Genital Human Papillomavirus (HPV) Reactivation patterns in Female Allograft Survivors Support HPV Vaccination**

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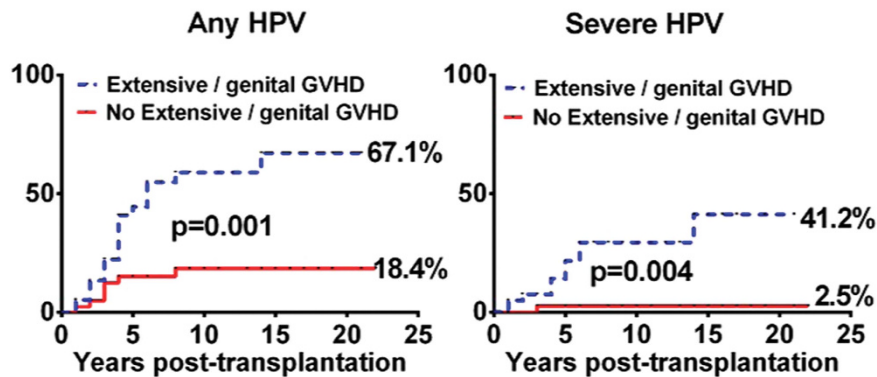
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**Introduction:** Human Papillomavirus (HPV) reactivation leading to cervical, vaginal, and vulvar warts and further progression to malignancy is an understudied issue for women after stem cell transplantation (SCT). Previous studies have focused on cervical dysplasia alone and identified graft versus host disease (GVHD) as a risk factor. In this analysis we incorporated pretransplant HPV status, latency of development, extent of disease and severity of HPV to determine underlying risk factors in the evolution of HPV disease to optimize screening and management.

**Material (or patients) and methods:** Gynecologic history and assessment were performed prospectively in this single-institute cohort of 82 female HLA-identical sibling SCT recipients. All recipients had survived  $> 1$  year and underwent serial cervical cytology and HPV testing with colposcopy and surgery as indicated. Prior HPV disease, marital status, age, ethnicity, diagnosis, transplant conditioning, genital GVHD (gGVHD), chronic GVHD (cGVHD) and immunosuppression treatment (IST)  $> 3$  years were incorporated into multivariate models for their association with occurrence, extent, persistence or severity of genital HPV disease. Backward stepwise logistic regression modeling was used for multivariate analyses.

**Results:** The median follow-up duration after SCT was 9.4 years, the median age was 36 years (range 10–68); acute leukemia was the most frequent diagnosis (45%); 93% of SCT were myeloablative ex-vivo T-lymphocyte depleted. The cumulative incidence estimates (Kaplan-Meier) of any genital HPV infection at 1, 3, 5, 10 and 20 years was 4.8, 14.9, 28.1, 36.7 and 39.7%, respectively. A high-risk HPV strain was found in 83% of informative subjects. 15 (18%) women had an abnormal pap prior to SCT, which was associated with risk of post-SCT HPV (OR = 6.5,  $P = 0.008$ ), and was the strongest risk factor for persistent HPV (OR = 23.2,  $P < 0.001$ ). 56 (68%) women had cGVHD (25 limited, 31 extensive); 21 (26%) had gGVHD. Having either extensive cGVHD or gGVHD was associated with increased risk of any HPV disease (OR = 5.7  $P = 0.002$ ) and a higher risk for severe dysplasia (CIN II-III / VIN

## Incidence of HPV



II-III; OR=13.1  $P=0.017$ ). 11 (13%) women underwent hysterectomy before or after transplant, which was associated with increased risk of multifocal HPV (OR=7.9  $P=0.01$ ).

**Conclusion:** Genital HPV disease rates were more than doubled in the setting of extensive cGVHD or gGVHD, likely reflecting HPV reactivation rather than new infection and implicates immune dysregulation. A history of prior HPV or the occurrence of either extensive cGVHD or gGVHD identifies women who deserve more frequent monitoring. The latency of HPV reactivation may be several years, supporting a window of opportunity for augmenting immunity through HPV vaccination.

**Disclosure of Interest:** None declared.

## O160

#### Risk Factors for Secondary Central Nervous System (CNS) Tumors in Survivors of Pediatric Hematopoietic Cell Transplantation

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**Introduction:** Survivors of Hematopoietic cell transplantation (HCT) are at risk of secondary solid tumors, including those of the central nervous system (CNS). The risk of solid tumors is known to increase over time and risk factors include radiotherapy and younger age. An increased risk of CNS tumors has also been shown in patients conditioned with Busulphan and cyclophosphamide without previous radiotherapy. This study aimed to determine the incidence and risk factors for developing CNS tumors in survivors of pediatric allogeneic HCT.

**Material (or patients) and methods:** We studied a large cohort of patients reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) who received an allogeneic HCT aged < 21 years old for both malignant and non-malignant conditions between 1976 and 2008. We included CNS malignancies diagnosed at least 1 year post transplant. To analyse the impact of pre-transplant therapy, especially radiotherapy, a case control design was used. Cases were matched on disease and duration of follow up. Controls were drawn from centers who had reported at least one case. Each case was matched with two controls.

**Results:** There were no cases of CNS tumors in the non-malignant cohort therefore this patient group were excluded from statistical analysis. There were 59 CNS tumors reported in 8661 patients transplanted for haematological malignancies.

CNS tumors occurred at a median of 7.5 years after HCT (range, 1year-28.8years). The majority of cases ( $n=53$ , 90%) received total body irradiation (TBI). Patients with CNS tumors had significantly lower 10 year overall survival when compared to the group as a whole (49% vs 72%;  $p=0.0006$ ) and the matched population (49% vs 90%;  $P<0.0001$ ). In comparison to the general population, pediatric HCT survivors had a 33 times higher than expected rate of CNS tumors (95% confidence interval, 22.98-45.77),  $P<0.0001$ .

On multivariate analysis significant risk factors for the development of a CNS tumor were having an unrelated donor HCT (HR 3.9), prior CNS disease (HR 3.72) and radiotherapy prior to conditioning (HR 2.13). There was no relationship with TBI dose.

**Conclusion:** Reassuringly, there were no CNS tumors patients transplanted for non-malignant conditions, the majority of whom received chemotherapy only conditioning. However, survivors of HCT for childhood haematological malignancies had a markedly increased risk of CNS tumors. The risks are increased in those who had unrelated donor HCT and in those who received pre-conditioning radiotherapy. This population should be advised of the risk and warrant lifelong surveillance for secondary CNS tumors.

**Disclosure of Interest:** None declared.

## O161

#### A single centre cohort report of long term clinical outcome of severe combined immunodeficiency following haematopoietic stem cell transplantation

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**Introduction:** Hematopoietic stem cell transplantation (HSCT) is curative for severe combined immunodeficiency (SCID), but the long-term sequel of pre-HSCT chemotherapy, HSCT and incomplete reconstitution are poorly documented. We aimed to explore the long-term clinical outcome of SCID survivor post-HSCT in a large single center cohort.

**Material (or patients) and methods:** A cross-sectional review of a post-HSCT SCID cohort attending the HSCT follow-up clinic at the Great North Children Hospital, Newcastle upon Tyne.

**Results:** 88/120 patients survived at latest follow up in 2015. The median follow up post-HSCT is 11.5 years (range, 2-27). 65 patients (74%) have on-going medical issues at last follow-up and 41 patients (47%) require on-going medication. 5 patients developed bronchiectasis [IL-7Ra (3) & IL-2RG SCID

(2)] and 1 has chronic pulmonary disease (IL-2RG SCID). All have normal lung function except for 1 with a major restrictive deficit (IL-7Ra SCID). 4 patients developed autoimmune hypothyroidism, 5 had autoimmune hemolytic anemia (2 resolved, 3 on-going). 11 patients (13%) have short stature (6 received myeloablative conditioning, 4 low toxicity myeloablative conditioning). 11 patients have hearing loss (50% ADA SCID). 8 have dental malformations - all received myeloablative conditioning. 4 patients have lymphoedema [IL-2RG SCID (3), JAK3 SCID (1)]. 12 patients have cutaneous papillomavirus infection (42% are IL-2RG SCID).

One patient developed chronic renal failure (Artemis SCID with myeloablative conditioning). 12/16 patients aged > 12 years have started menses and 38 (86%) > 13 years have achieved puberty. 9 patients experienced neurocognitive problems: learning difficulties (4), Attention Deficit Hyperactive Disorder (2) and one patient each for Autism Spectrum Disorder, Cerebral Palsy, Delayed Development and Low Mood.

64 (74%) have discontinued immunoglobulin replacement therapy (78% received conditioning prior to transplant). Antibodies levels to pneumococcal serotypes are only available for 51 patients and 45% of patients have protective antibody levels towards more than 9 pneumococcal serotypes.

**Conclusion:** Survival outcome is good, but a significant number experience on-going medical issues which requires treatment and monitoring. Further studies are needed to improve the long-term outcome for these patients.

**Disclosure of Interest:** None declared.

**O162 Autologous transplantation in older myeloma patients: is it worth the effort?**

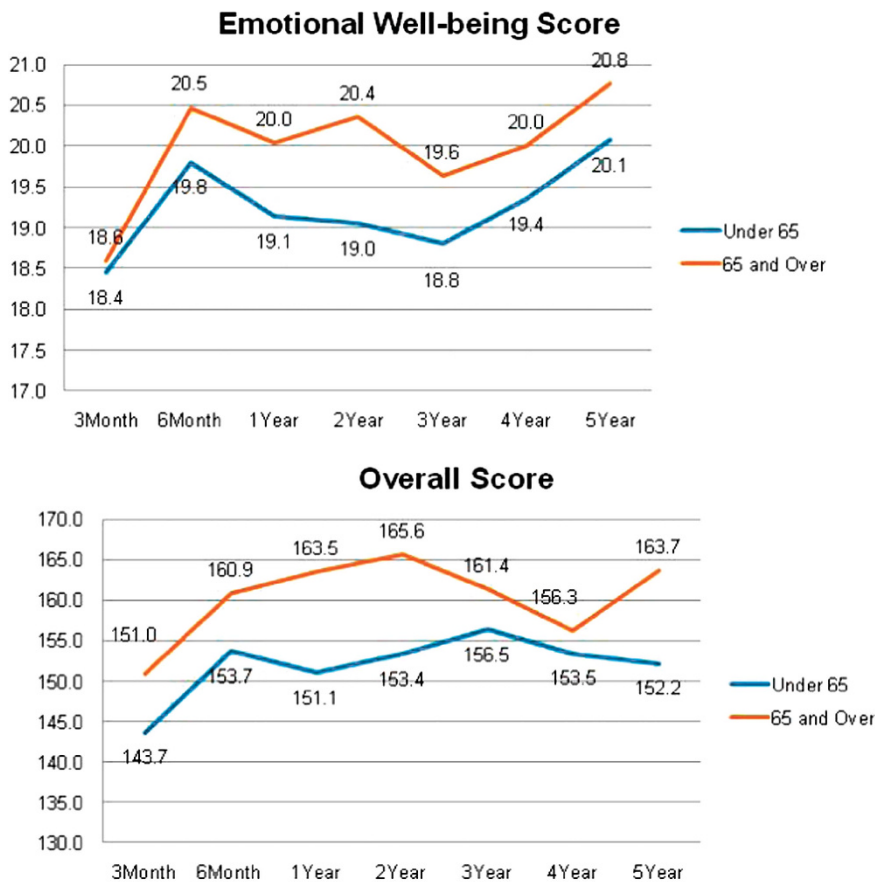
K. Wilson<sup>1,\*</sup> on behalf of South Wales Blood and Marrow Transplant Programme, D. Davies<sup>1</sup>, S. Thompson<sup>1</sup>, T. Hywel<sup>2</sup>, J. Bridgeman<sup>3</sup>, T. Banks<sup>3</sup>, S. Al-Ismaïl<sup>2</sup>, H. Sati<sup>2</sup>, W. Ingram<sup>1</sup> on behalf of South Wales Blood and Marrow Transplant Programme <sup>1</sup>Clinical Haematology (BMT Programme), UNIVERSITY HOSPITAL OF WALES, Cardiff, <sup>2</sup>Clinical Haematology (BMT Programme), Singleton Hospital, Swansea, Swansea, <sup>3</sup>Tenovus Cancer Care, Cardiff, United Kingdom

**Introduction:** Multi-agent chemotherapy followed by consolidation with an autologous stem cell transplant is standard therapy for fit patients with myeloma in first plateau. This approach is controversial in older patients due to the lack of a consistent survival benefit. Given that myeloma in plateau phase spares the patient from complications and affords a treatment-free interval, BMT may still be beneficial in older patients, especially if recuperation is not unduly prolonged. We therefore compared quality of life post transplant in older and younger patients with myeloma.

**Material (or patients) and methods:** The database of the SWBMT Programme was interrogated to retrospectively identify myeloma patients who were transplanted between June 1994 and October 2015. From 2011, FACT-BMT (version 4) questionnaires were prospectively administered at 3, 6, 12 months and annually thereafter. We compared quality of life in older (age ≥ 65 years) and younger (< 65 years) patients over a 5-year period.

**Results:** During the study period 433 patients were transplanted. The older cohort was 67 years (range 65-76, n = 102)

[O162]



and the younger cohort 57 years (range 28-64,  $n=331$ ) old. There was a male preponderance in both groups (67% older, 60% younger cohort) and Durie-Salmon and ISS stages were similar. The older cohort was transplanted later (median year of transplant 2011 versus 2009) and full dose melphalan (200 mg/m<sup>2</sup>) was delivered to 92% and 89% of the older and younger cohorts, respectively. Karnofsky performance score was  $\geq 90\%$  in 81% and 73% of older and younger patients. Median EBMT risk score and HCT-CI were similar, being 3 and 1, respectively, for both groups. 55% of older and 53% of younger patients had a co-morbidity. Median follow up for surviving patients was 29 and 37 months for the older and younger cohorts with 74% and 57%, respectively, being alive. Progression free survival (18 months versus 20 months) and non relapse mortality (3.9% versus 3.6%) was similar for the older and younger cohorts. For both overall quality of life score as well as each of the five individual domains: Physical well-being, Social/Family well-being, Emotional well-being, Functional well-being and Additional concerns, the older cohort scored higher at each of the time points assessed over the five year period.

**Conclusion:** In this large cohort of 433 patients transplanted over a 21-year period, older patients with myeloma fared no worse than their younger counterparts with regards to progression free survival or toxicity. Although the majority of older patients received full dose melphalan conditioning, self-declared quality of life was better than that of the younger cohort. In light of the fact that myeloma remains incurable, maintaining quality of life in surviving patients is of paramount importance. This study shows that autologous transplantation does not have a deleterious effect on recuperation in fit older patients with myeloma and suggests that this treatment should not be denied solely on the basis of age.

**Disclosure of Interest:** None declared.

#### O163

##### Measles, Mumps, Rubella (MMR) and Varicella Immunity Post-Allogeneic HSCT

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**Introduction:** Guidelines published in 2009, reviewed in 2012, recommend MMR and to consider Varicella vaccination at a minimum of 24 months post HSCT, in patients at least 12 months off immunosuppression and with no GVHD. In WBMTU we elected not to routinely vaccinate any patients with MMR or Varicella in view of the complexity in determining the appropriate time for vaccination, especially in the community by non-specialists. We check MMR and Varicella serology post transplant and use this information to determine who requires vaccination in association with specific risk factors.

**Material (or patients) and methods:** Between 01/03/02 – 28/02/14 we performed 383 Allogeneic stem cell transplants (SCT) on 363 patients of which 110 have been evaluated with MMR +/- Varicella serology. 59% were unrelated donor SCT and 45% received reduced intensity conditioning (RIC). 93% of all SCT received Alemtuzumab at doses of 20–50mg. Patients were transplanted for the following indications: CLL/Lymphoma 21, Myeloma 6, AML/ALL 60, MDS/MPD/CML 20, SAA 3.

#### Results:

Serology Result	VUD	Sib	MAC	RIC	Time after SCT < 2 yrs	Time after SCT 2-5 yrs	Time after SCT > 5 yrs
Mu+Me+R+	27	20	23	24	9	27	11
Mu+ Me+R-	4	4	5	3	1	2	5
Mu+ Me- R-	2	1	2	1			3
Mu-Me+ R+	13	5	10	8	1	12	5
Mu- Me- R+	7		4	3	2	4	1
Mu- Me+ R-	1	3	2	2		2	2
Mu+ Me-R+	2	3	2	3		2	3
Mu- Me- R-	9	9	13	5	2	5	11
Total (%)	65 (59)	45 (41)	61 (55)	49 (45)	15 (14)	54 (49)	41 (37)

Mu – mumps; Me – measles; R - rubella

MMR	Serology	Total
Triple	positive	47/110 (43%)
Double	positive	31/110 (28%)
Single	positive	14/110 (13%)
Triple	negative	18/110 (16%) (2/110 1.8% with borderline / low total IgG)
Non-Alemtuzumab	MMR	Serology
Triple	positive	2/8 (25%)
Double	positive	2/8 (25%)
Single	positive	1/8 (12.5%)
Triple	negative	3/8 (37.5%)
Triple Positive Patients		

9/47 (19%) positive by two years post SCT and 36/47 (77%) positive by 5 years.  
 Triple Negative Patients

72% received myeloablative conditioning (MAC), of which 62% received total body irradiation.  
 61% remain negative at five years post SCT

#### Positive MMR serology by virus

Measles	77/110 (70%)
Mumps	61/110 (55%)
Rubella	77/110 (70%)

#### Disease

5/6 (83%) of myeloma patients were triple negative. There were no other disease related trends.

#### Varicella Serology

Positive 58/76 (76%); 41/58 (71%) positive by 5 years and 55/58 (95%) positive by 10 years post SCT

#### Age at Transplant:

Due to the small numbers of SCT in patients >60 years (14/110, 13%) in this series, there is no significant difference in serology results compared to patients < 60 years

**Conclusion:** 92/110 (84%) of patients show serological evidence of immunity to at least one virus, 70% specifically showing measles immunity. Reduced positive serology for Mumps may reflect a lower incidence of disease or vaccination within the donor population. Further testing of donor samples to confirm this observation is planned.



High rates of Varicella positivity demonstrated. Use of Varicella vaccine appears unjustified, although positive serology alone does not prevent Varicella Zoster re-activation in our practice. Contrary to expectation patients receiving MAC show higher rate of triple negative serology compared to RIC. The use of Alemtuzumab within the conditioning schedule does not appear to impact on post-SCT antibody production, even within the first two years. This potentially justifies our local vaccination policy.

**Disclosure of Interest:** None declared.

**O164**

**Long-Term Prognosis of One-Year Survivors of CD34-Selected Allogeneic Hematopoietic Stem Cell Transplantation: A Landmark Analysis**

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**Introduction:** CD34-selected allogeneic hematopoietic cell transplant (CD34 allo-HCT) significantly reduces GVHD and is under study in a multicenter phase 3 trial in the US (BMT CTN 1301, NCT02345850). Yet precise information about long-term prognosis for patients who receive CD34 allo-HCT and survive without relapse past the early post-HCT period is lacking.

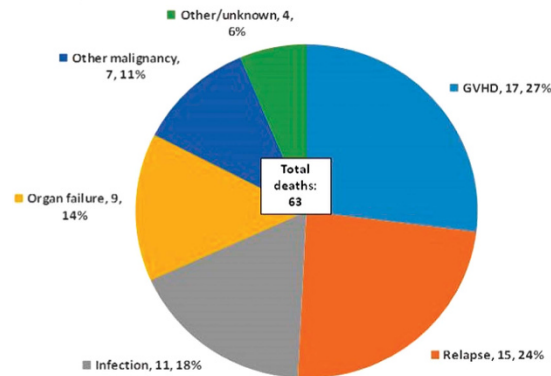
**Material (or patients) and methods:** This is a single-center landmark analysis in recipients of CD34 allo-HCT for AML, MDS, or ALL alive and relapse free 1 year post-HCT. We estimated OS and RFS by Kaplan-Meier methods. Relapse and non-relapse mortality (NRM) were competing events and estimated with cumulative incidence functions. Association of variables with RFS/OS was evaluated using Cox regression; those significant to  $p \leq .05$  were evaluated in a multivariable Cox regression model.

**Results:** Of 421 recipients of CD34 allo-HCT for AML/MDS/ALL from 2/2000 through 6/2013, 276 were alive/disease free at 1 year (study cohort), 164 (59%) with AML, 79 (29%) with MDS, and 33 (12%) with ALL. Median age was 54 years (range 18-72). Of those with AML, 116 (71%) received allo-HCT in CR1, 40 (24%) in CR2 or later, and 8 (5%) in PR or refractory disease. In MDS, 13 (84%) were in CR pre-HCT; the remainder had residual MDS. In ALL, 27 (82%) were transplanted in CR1, 5 (13%) in CR2 or later, and 1 (3%) with refractory leukemia. HCT-Comorbidity Index was 0 for 72 patients (26%), 1-2 for 90 (33%), and  $\geq 3$  for 114 (41%). All received ablative conditioning, 105 (38%) with a TBI-based regimen and 171 (62%) chemotherapy-based. One hundred seven patients (39%) received grafts from MRDs, 91 (33%) from MUDs, 9 (3%) from MMRDs, and 69 (25%) from MMUDs. At median 5 years follow-up from 1 year post-HCT, 5 year OS and RFS were 77% and 73%, and cumulative incidence of relapse and NRM 10% and 17%. Estimated OS/RFS and cumulative incidence of relapse/NRM are shown in the table. In AML/MDS, RFS/OS did not vary significantly with disease status; in ALL, patients transplanted in CR1 saw significantly enhanced RFS vs. CR2+/refractory disease (HR 0.22, 95% CI .07-.71;  $P = .01$ ), with OS marginally significant (HR .29, 95% CI .08-1.07,  $P = .06$ ). In multivariable analysis, HCT-CI  $\geq 3$  was associated with significantly increased risk of death (HR 2.23, 95% CI 1.13-4.41,  $P = .01$ ) and marginally significant increased risk of relapse/death (HR 1.57, 95% CI .87-2.83,  $P = .06$ ). A total 63 patients died after the landmark, with GVHD the most common cause (figure), followed by relapse and infection. Of the 15 patients who died of relapse, 9 relapsed in the first 2 years after the landmark.

TABLE: Estimated Outcomes for 1-Year Disease-Free Survivors of CD34 Allo-HCT

	5 yr OS (95% CI)	5 yr RFS (95% CI)	5 yr cum. inc. Relapse (95% CI)	5 yr cum. inc. NRM (95% CI)
Overall	77% (71-82)	73% (67-78)	10% (6-14)	17% (12-21)
AML	79% (71-85)	77% (69-83)	9% (5-14)	14% (8-20)
MDS	76% (64-85)	72% (59-81)	10% (3-18)	18% (9-27)
ALL	72% (53-84)	61% (42-76)	14% (1-27)	25% (10-40)

Figure: Causes of Death in Recipients of CD34 Allo-HCT Alive and Relapse Free at 1 Year Landmark



**Conclusion:** Patients alive and relapse free 1 year post-CD34 allo-HCT have an excellent prognosis, though NRM risk particularly from GVHD persists, while the impact of disease-related risk factors and relapse declines.

**Disclosure of Interest:** None declared.

**O165**

**ADOPTIVELY TRANSFERRED HAPLOIDENTICAL NK CELLS AGAINST REFRACTORY MDS, HIGH-RISK MDS AND REFRACTORY AML AS A BRIDGE TO TRANSPLANTATION. COMPLETE REMISSION ASSOCIATED WITH DETECTABLE NK CELLS**

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**Introduction:** We here report from our recently closed phase I/II study of HLA-haploidentical NK cell therapy to patients with high-risk myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) not eligible for standard therapies. The preparative regimen consisted of intermediate doses of Cyclophosphamide (Cy), Fludarabine (Flu) and titrated doses of total lymphoid irradiation (TLI). The trial design excluded systemic IL-2 treatment to avoid expansion of regulatory T cells and to test if *in vivo* expansion could be obtained without IL-2 support.

**Material (or patients) and methods:** 16 patients were treated with Cy/Flu and an escalating dose of TLI (2 Gy and 4 Gy), followed by infusion of short-term IL-2 activated (16 hours) NK cells. Seven patients received daily cyclosporine A after the conditioning. Three had relapsed chemotherapy refractory primary AML, ten had secondary relapsed or refractory MDS-AML and three had high risk MDS with fibrosis.

**Results:** The treatment was well tolerated and no severe non-infectious toxicity could be observed in the patients. The endpoint of NK cell expansion was not reached, but nine patients had positive microchimerism (NK cells of donor origin detectable by RT-PCR at day 7-14), that thereafter became undetectable within 7-14 days. Five of nine patients achieved complete remission (CR) after 1 month. Five patients became eligible for and proceeded to allogeneic stem cell transplantation (SCT). Four of these are still free from disease 8-23 months after transplantation. No patient without detectable NK cells obtained CR. Six patients died from progressive disease and three patients with minor response and progressive disease died in infections within three months of therapy.

**Conclusion:** The results suggest that a combined lymphodepletive regimen followed by NK cell therapy may induce remission in patients with high-risk, chemo-refractory disease and provide a bridge to allogeneic stem cell transplantation. Notably, clinical responses were observed after only a minimal *in vivo* NK cell expansion and were independent of KIR-ligand mismatch. Based on these results we are planning to start a multi-center study with NK cell therapy, as a bridge to SCT, against high-risk and azacytidine refractory MDS. Immunological and molecular studies of the disease as well as the reconstituted cells are ongoing and will also be presented.

**Disclosure of Interest:** None declared.

**O166**

**A new strategy to control cd19 car expression; regulation under tetracycline-inducible system allows on-demand CD19 car expression and disappearance**

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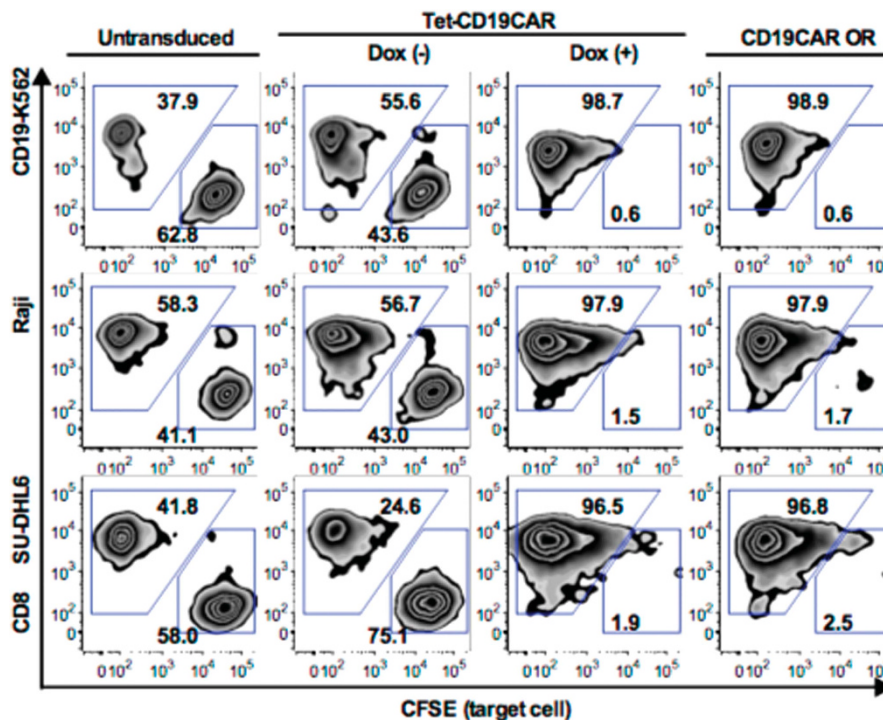
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**Introduction:** Clinical trials of CAR-T therapy demonstrated toxicities such as on target/off tumor effects, which could be life-threatening. These toxicities are major drawbacks for broader application of CAR-T therapy. Therefore, a further modification to control CAR expression is needed. In this study, we aimed to develop inducible CAR-T cells based on the use of Tet-on system.

**Material (or patients) and methods:** We developed inducible CD19CAR system by infusing anti-CD19-CD3ζ-CD28-tEGFR into pRetroX-TetOne vector (Tet-19CAR). By using Tet-19CAR transduced SUPT1 cells, the expression and disappearance kinetics of CAR were determined. We also retrovirally transduced Tet-19CAR into human CD8+ T cells, and achieved more than 90% purity of CAR positive T cells after beads selection. These CAR-T cells were used *in vitro* assays such as cytotoxicity, cytokine production and T-cell proliferation. Furthermore, NOG mice bearing CD19+ Raji cells were given Tet-19CAR with or without doxycycline (Dox) and examined for their overall survivals and tumor fluxes with bioluminescence imaging.

**Results:** With more than 100 ng/mL of Dox, CD19CAR was expressed in both of SUPT1 and CD8+ T cells. For maximum and minimum expression, 24 and 72 hours of incubation

[0166]



CAR-T cells and CFSE-labeled CD19-K562, Raji or SU-DHL6 were cultured at a 1:1 ratio without IL-2 supplementation for 96 hr. The percentage of surviving CAR-T cells and residual various cell lines within the live cell gates are shown. Data are representative of three independent experiments using three independent Tet-CD19CAR T cell lines.

periods were needed after an addition and a removal of Dox, respectively. To determine the cytotoxicity of Tet-19CAR-T cells, we performed <sup>51</sup>Cr release assay and coculture assay against CD19-K562. Dox(+) Tet-19CAR showed an equal lytic activity to conventional CD19CAR-T cells (c19CAR). In contrast, Dox(-) Tet-19CAR exhibited significantly lower cytotoxicity against CD19+ target cells [Dox(-) Tet-19CAR, Dox(+) Tet-19CAR and c19CAR: 14.0 ± 4.0%, 38.0 ± 4.0% and 37.0 ± 2.0% at an E:T ratio = 10:1, respectively]. In the coculture assay, Dox(+) Tet-19CAR eradicated CD19-K562, while they failed to suppress the target cells without Dox (figure). In the intracellular IFN-γ assay against CD19-K562, a similar proportion of responder was IFN-γ+ in Dox(+)Tet-19CAR and c19CAR. In contrast, a significantly low proportion of IFN-γ+ cells was observed in Dox(-)Tet-19CAR [Dox(-)Tet-19CAR 1.0% ± 0%, Dox (+)Tet-19CAR 19.1% ± 6.0% and c19CAR 21.5% ± 4.0%]. Similar to intracellular IFN-γ assay, ELISA revealed that Dox(+)Tet-19CAR and c19CAR produced IL-2 and IFN-γ equally well. However, Dox(-)Tet-19CAR hardly did. [IL-2 (ng/ml): Dox(-)Tet-19CAR, 1.00 ± 0.060, Dox(+)Tet-19CAR, 9.25 ± 0.30 and c19CAR 8.75 ± 0.68]. We next analyzed CAR-T cell proliferation upon stimulation with CD19-K562 over 96 hours. Dox(+)Tet-19CAR showed 6-7 fold expansion, whereas Dox(-)Tet-19CAR failed to proliferate. Regarding *in vivo* experiment, the mice treated with c19CAR or Dox(+)Tet-19CAR showed significantly improved survival as compared with Dox(-)Tet-19CAR (*n* = 8, *P* = 0.003, log-rank test). Furthermore, the mice given c19CAR or Dox(+)Tet-19CAR demonstrated significantly lower tumor burden than Dox(-)Tet-19CAR on the 21th day of infusion.

**Conclusion:** We generated tetracycline-inducible CAR-T cells and successfully controlled the CAR expression with Dox administration. Dox(+)Tet-19CAR effectively lysed CD19+ target cells, secreted cytokines and proliferated upon CD19+ cell stimulation *in vitro*. Moreover, Dox(+)Tet-19CAR also showed robust their anti-tumor abilities in a xenograft model. On the other hand, Dox(-)Tet-19CAR lost their abilities to behave as CAR-T cells both *in vitro* and *in vivo*.

**Disclosure of Interest:** None declared.

#### O167

##### Low-dose infusions of CD45RA-depleted donor lymphocytes to improve immune reconstitution after TCR alpha/beta-depleted transplantation – results of a pilot trial

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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. In this pilot trial we have tested the hypothesis that infusions of low dose memory (CD45RA-depleted) T-cells are safe and may provide effective means to control viral infections before recovery of broad repertoire of T-cells.

**Material (or patients) and methods:** Fifty six patients received TCR-alpha/beta depleted transplants, median age was 10y (0-22), m/f-37/19, with malignant (n-37) and non-malignant (n-19) disorders after either 1<sup>st</sup> (n-52) or 2<sup>nd</sup> (n-4) HSCT. Donors were haploidentical (n-23) or matched unrelated (n-33). Patients were eligible if they had stable graft function, no signs of active GVHD or severe infection. Only CMV seropositive donor/recipient pairs were eligible. Infusions of memory T-cells were planned at escalating doses (25x10<sup>3</sup>/kg, 50x10<sup>3</sup>/kg, 100x10<sup>3</sup>/kg in haploidentical, and 100x10<sup>3</sup>/kg,

200x10<sup>3</sup>/kg, 300x10<sup>3</sup>/kg, in MUD transplants), with monthly intervals. T-cells were derived from G-CSF stimulated (n- 39) or unstimulated (n-17) apheresis of the original donors. Apheresis product was processed with single-step CD45RA depletion procedure on CliniMACS Plus or Prodigy (n - 56) instrument. CD45RA-depleted fraction was aliquoted and cryopreserved for further use. Beyond routine monitoring of CMV, EBV and Adeno DNA load, lymphocyte subset regeneration and hematopoietic chimerism, development of pathogen-specific (CMV, EBV, Adeno) immunity was monitored in selected cases by the ELISPOT assay for IFN-gamma production in response to respective antigen stimulation.

**Results:** Between 23.04.14 and 15.10.2015, 56 patients received 143 memory T-cell infusions. Final product contained negligible numbers of CD45RA+ naive T-cells. Median day of the first infusion was + 49 (0-498). Sixteen patients had CMV viremia at the moment of 1<sup>st</sup> DLI. Five patients developed signs of acute (n-4) or chronic (n-1) GVHD after infusion. At median follow-up of 254(34-430) days the cumulative incidence of any GVHD was 9% (95% CI: 4-21). Three patients had had acute GVHD prior to 1<sup>st</sup> memory DLI. In a group of 53 patients without prior GVHD CI of GVHD at +200 FU was 4% (95% CI: 1-16). In a group of 56 patients CI of TRM was 5% (95% CI: 1-18). Causes of death included adenovirus (n-2) and multiorgan failure (n-1). Of note, 5 of 34 patients with CMV reactivation were able to clear CMV without pharmacological intervention. According to Elispot analysis, median number of CMV-reactive cells in the peripheral blood increased significantly from baseline: median 1 (0-972) to 360 (0-983) cells per 3x10<sup>5</sup> MNC, *P* < 0,01.

**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 after the T-depleted haploidentical and unrelated transplantation. A prospective trial of preventive infusions of donor memory cells is planned.

**Disclosure of Interest:** None declared.

#### O168

##### Safety and Efficacy of CD19 Chimeric Antigen Receptor-Modified T Cells for Relapsed/Refractory Acute Lymphocytic Leukemia Under Fludarabine and Cyclophosphamide based Lymphodepletion

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**Introduction:** Patients with relapsed and refractory B cell ALL have a dismal prognosis. Chemotherapy followed by autologous T cells that are genetically modified to express a CD19-specific chimeric antigen receptor (CD19CAR) has shown promise as a novel therapy for these patients; however, the risk of severe cytokine release syndrome (CRS), neurotoxicity and relapse has tempered enthusiasm for widespread application of this approach. We are conducting a phase I clinical trial to evaluate the safety and efficacy of CD19CAR T cells in patients with relapsed or refractory CD19+ ALL.

**Material (or patients) and methods:** Refractory or relapsed CD19+ ALL patients were enrolled. Eligible patients underwent leukapheresis, and T cells were transduced with a Lenti-virus encoding a CAR construct composed of anti-CD19 scFV linked to 4-1BB and CD3ζ signaling domains. To enhance the engraftment of transferred CAR T cells, patients received fludarabine and cyclophosphamide based lymphodepleting chemotherapy followed by infusion with 1x10E6-10x10E6 CAR T cells/kg, given over a period of 3 consecutive days.

**Results:** 11 pts were enrolled. The median age was 42 years (range, 26-57). 3 pts (27%) had Ph+ B-ALL (T315I mutation in 2 pts), 5 pts (45%) had prior allogeneic hematopoietic stem cell

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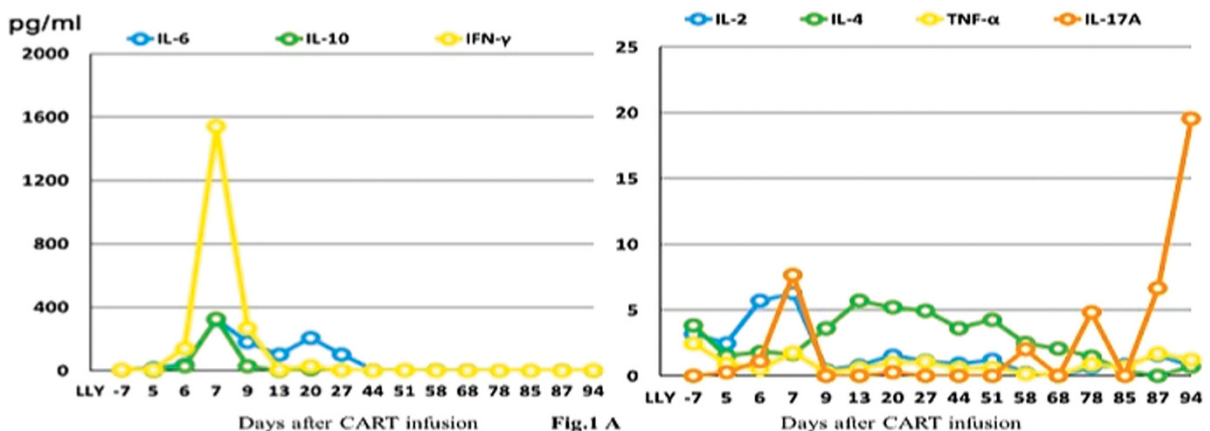


Fig.1 A

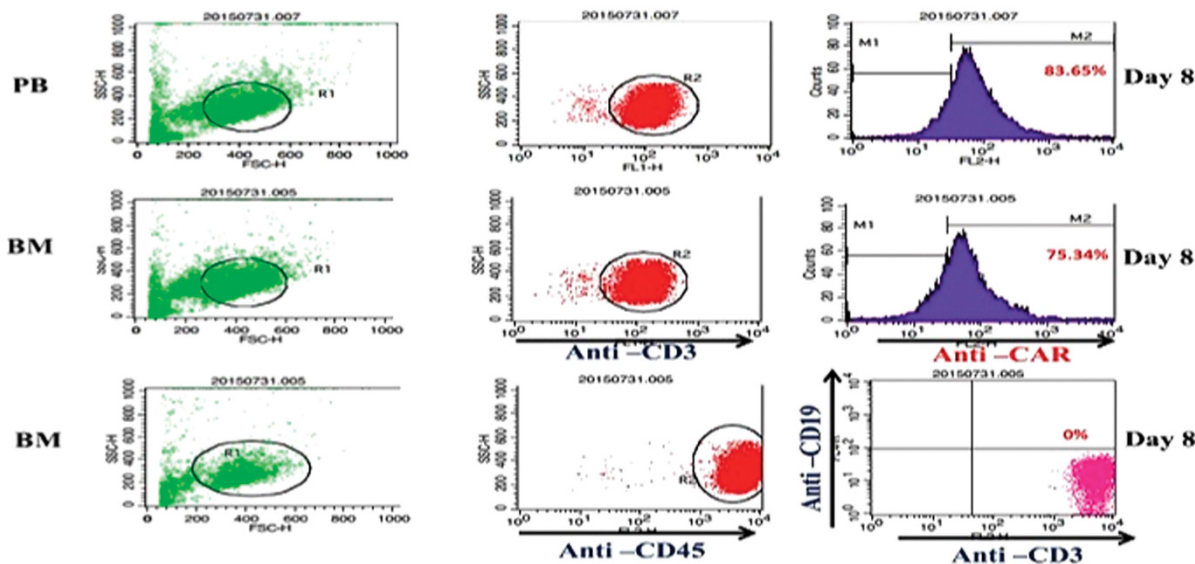


Fig.1 B

transplant (allo-HSCT), and 6 pts (55%) had 3 or more prior lines of ALL therapy before receiving the CART cell therapy. There were no infusion toxicities. 2 pts died of infection 5 and 11 days after CART infusion respectively and didn't evaluate primary disease status. 9 of 10 patients (90%) achieved a CR 7-10 days after CART cell infusion (Table 1) while 1 got partial remission (8.75%). All the patients (100%) achieved a CR 1 month after CART infusion. Severe CRS associated with elevated serum IFN- $\gamma$  and IL-6 was observed in patients with a high tumor burden (Fig.1A). Treatment for CRS was rapidly reversed in all cases with the IL6-receptor antagonist tocilizumab, together with corticosteroids in 1 pt. CART cells expanded *in vivo* and could be detected in blood and bone marrow (Fig.1B). With median follow up 62 days (32-140), 4 pts have ongoing CR. 5 pts with a CR at 1month have subsequently relapsed, 2 with CD19(-) blasts.

**Conclusion:** CD19 CART cells can undergo robust in-vivo expansion and can induce CR in pts with relapsed and refractory ALL. This approach also has promise as a salvage therapy for patients who relapse after allo-SCT. CD19 CART therapy is associated with a significant CRS that responds rapidly to IL-6-targeted anti-cytokine treatment. However, relapse still remains the main obstacle for the successful clinical adaptation. Mechanisms and treatment strategy of relapse after CART infusion needs further study. These findings will also need to be evaluated and confirmed in a larger phase 2 trial.

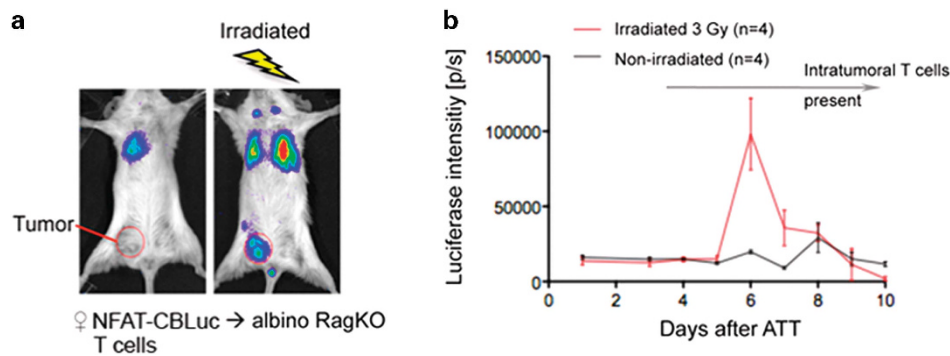
**Disclosure of Interest:** None declared.

**O169**  
**Generation of a dual-luciferase transgenic mouse model for concurrent visualization of trafficking, accumulation and activation of adoptively transferred T cells**

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**Introduction:** Trafficking of tumor-specific T cells to the tumor, their infiltration into the tumor tissue and their activation at tumor site are essential for efficient tumor eradication via adoptive T cell therapy (ATT). Beside the poor knowledge of cancer-driving antigens immune escape mechanisms and local immunosuppression in the tumor microenvironment are limiting the success of ATT. Furthermore, most targetable tumor antigens are not exclusively expressed by tumor, so that adoptively transferred T cells frequently attack nonmalignant host tissue resulting in possibly devastating graft-versus-host-disease.

**Material (or patients) and methods:** We have generated a transgenic mouse expressing an NFAT (Nuclear Factor of Activated T cell)-dependent Click-beetle luciferase (NFAT-CBLuc mouse). After crossing NFAT-CBL mice with constitutive Renilla Luciferase-transgenic B6 mice, we are able to monitor



migration and activation of adoptively transferred T cells *in vivo*. We are using these mice to optimize migration and effector function of adoptively transferred T cells in an H-Y tumor model via screening for combination treatments capable of overcoming immunosuppressive mechanisms in the tumor microenvironment.

**Results:** In our first experiments using our bioluminescent reporter mouse model, we have transferred sorted T cells from female NFAT-CBLuc mice, which were primed *in vivo* against H-Y by male splenocytes, and injected them into albino-RagKO recipients. One group of recipients received additionally sublethal irradiation four hours before T cell transfer. We could show increased T cell tumor infiltration and T cell activation at tumor site upon sublethal irradiation of the recipients prior to ATT (Figure 1a). The combination with irradiation improved the efficacy of ATT, possibly mediated by increased tumor-antigen presentation after irradiation-induced inflammation. Interestingly, the activation signal peaked around day 6 after transfer before rapidly declining to base-line levels although T cells remained in the tumor, illustrating the local suppression of immune cells within the tumor (Figure 1b).

**Conclusion:** In summary, we established a bioluminescent reporter-transgenic mouse model allowing detailed analysis of longitudinal *in vivo* T-cell trafficking and local activation. This novel tool could be applied in different tumor models in order to develop and evaluate novel therapeutic strategies for optimal tumor-specific ATT overcoming immune suppression by the tumor and limited on-target/ off-tumor toxicity.

**Disclosure of Interest:** None declared.

#### O170

##### Wilms Tumor 1 specific T-cell receptor gene-modified autologous lymphocytes displayed a transient clinical response in a patient with acute myeloid leukemia

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**Introduction:** The prognosis for acute myeloid leukemia (AML) with myelodysplasia in aged patients is poor, because of high mortality related to intensive chemotherapy and allo-HSCT. Thus, additional treatment options for this population are needed. In this context, we are conducting a phase I clinical trial of adoptive immunotherapy using T-cell receptor (TCR) gene-modified T cells targeting leukemia-associated antigen Wilms Tumor 1(WT1) for the treatment of refractory AML and high risk myelodysplastic syndrome (MDS).

**Material (or patients) and methods:** Protocol of this clinical study is approved by our institutional review board (UMIN0001159). A 69 y/o male patient with AML with multilineage dysplasia (AML/MLD) participated in this phase I study after obtaining written informed consent under the declaration of Helsinki. Lymphocytes from 100 ml of patient's peripheral blood (PB) had been sent to the cell processing center, and gene-modified to express HLA-A\*24:02-restricted and WT1<sub>235-243</sub> nonamer epitope-specific TCR using the specially designed retrovirus vector encoding codon-optimized WT1-specific TCR a/b genes and siRNAs complementarily inhibiting constant regions of endogenous TCR a/b genes (WT1-siTCR vector). After expansion and quality-check, frozen gene-modified lymphocytes were sent back to Ehime University hospital. According to the study protocol,  $2 \times 10^8$  total cells (cohort 1) composed of 29% CD4, 76.6% CD8 including 11.6% HLA\*2402/WT1 tetramer-positive subset were infused twice 4 weeks apart. 2 days after 2<sup>nd</sup> infusion, WT1 peptide vaccination was added twice 2 weeks apart. IL-2 support was not employed. Disease status, kinetics of infused lymphocytes and immunological examinations were serially conducted for 56 days after 1<sup>st</sup> infusion.

**Results:** Eventually no severe adverse event associated with infused gene-modified lymphocytes was observed. Infused gene-modified lymphocytes in PB assessed by DNA-PCR were detectable for 2 weeks after each infusion. At 1<sup>st</sup> infusion, leukemia blasts (LB) in bone marrow (BM) was 24.8%. At 2<sup>nd</sup> infusion, LB in BM was 47.1%. Three weeks after 2<sup>nd</sup> infusion, LB in BM decreased to 18.6% accompanied with improved PB cell counts (partial remission), and the patient became independent of RBC transfusion. Simultaneously, transient elevation of CRP and blood uremic acid concentration were observed, and copy number of WT1 mRNA in PB returned to baseline. Large granular lymphocytes became noticeable durably in PB after 2<sup>nd</sup> infusion. Additionally, serial examination of serum IgG protein array revealed the epitope-spreading derived from destroyed LB. This clinical response lasted longer than 2 months. Even after disease progression, AML/MLD was successfully managed simply using 2 courses of low-dose chemotherapy. 13 months after 2<sup>nd</sup> infusion, at this presentation, this patient still remains an out-patient, free from transfusion and has ECOG performance status 0. No clonal evolution of gene-modified lymphocytes developed.

**Conclusion:** Although small number of gene-modified T cells were infused, in this case, WT1-siTCR-T cells were safely administered and displayed a transient inhibition of leukemia. Secondary immune response against leukemia was induced. Finally, limited clinical efficacy seemed attributable to the limited persistence of gene-modified T cells *in vivo*.

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S. Okamoto Employee of: Takara Bio Inc., J. Mineno Employee of: Takara Bio Inc., H. Nishikawa: None declared, H. Shiku Funding from: Takara Bio Inc., M. Yasukawa: None declared.

#### O171

##### **NY-ESO-1 TCR single edited t cells to treat multiple myeloma without inducing GvHD**

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**Introduction:** Transfer of T cell receptors (TCR) specific for tumor-associated antigens is a promising approach for cancer immunotherapy. The TCR gene editing technology, based on the knockout of the endogenous TCR alpha and beta genes, followed by the introduction of tumor-specific TCR genes, proved safer and more effective than conventional TCR gene transfer. While successful, complete editing requires multiple manipulation steps and four transduction procedures.

**Material (or patients) and methods:** To reduce the duration and complexity of the procedure, we developed and tested the 'single TCR editing' (SE) approach, based on the disruption of the endogenous TCR alpha chain only, followed by the transfer of genes encoding for a tumor specific TCR. We validated SE exploiting an HLA-A2 restricted TCR specific for NY-ESO-1<sub>157-165</sub>, a cancer testis antigen expressed by numerous solid tumors and by a large proportion of high-risk multiple myeloma plasmacells. Conventional TCR gene transfer (TR) and SE cells were compared in terms of efficacy and safety *in vitro* (phenotypical analysis, gamma-IFN ELISPOT, <sup>51</sup>Cr release and co-culture assays) and *in vivo* (adoptive T cell transfer in an experimental humanized-mouse model of multiple myeloma).

**Results:** The SE protocol rapidly produced high numbers of tumor specific T cells, with an early differentiation phenotype. When tested *in vitro*, single edited T cells showed a high killing activity against multiple myeloma, similar to that of T cells redirected with TR; however, while TR cells proved highly alloreactive, SE cells showed a favorable safety profile. When infused in NSG mice previously engrafted with myeloma, SE cells mediated tumor rejection without inducing xenogeneic graft versus host disease (GvHD), thus promoting a significantly higher survival than that observed in mice treated with TR cells. The detrimental alloreactive effect mediated by TCR transferred T cells, which led to a xenogeneic GvHD rate comparable to that of untransduced T cells, was confirmed by histopathological examination, that revealed TR cell infiltrations and GvHD-like lesions in several murine organs and tissues. Conversely, the full abrogation of the endogenous TCR repertoire in the SE tumor redirected lymphocytes prevented any off-target reactivity that normally leads to GvHD in mice, potentially providing a safer T cell therapy against cancer.

**Conclusion:** Overall, the single TCR gene editing procedure provides a rapid and efficient method for generating primary T cells that highly express a tumor specific TCR and are permanently devoid of their endogenous TCR repertoire, and thus represents a protean platform for the effective and safe adoptive transfer of allogeneic and autologous T lymphocytes redirected towards any desired tumor associated antigen.

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