

# ABSTRACTS FROM THE 43RD ANNUAL MEETING OF THE EUROPEAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION: PHYSICIANS—POSTERS SESSION (P001-P738)

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## Hematopoietic stem cells

### P001

#### Absolute immature platelet count may predict the platelets graft in patients undergoing ALLO-HSCT

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The immature platelet fraction (% IPF) is a relatively new parameter that measures young (reticulated) platelets in peripheral blood (PB). IPs rise as bone-marrow (BM) production of platelets increases. Several clinical utilities of the %IPF have been already proved, as the treatment response monitoring in aplastic anemia or immune thrombocytopenic purpura. In this study, we aimed to found if IP measurement might be useful during the grafting phase of HSCT. This study includes 141 patients who underwent allo-HSCT in our center during the last 2.5 years. 79 were male (56%) and 62 female (44%). Median age was 52 years (range: 7–69). Baseline diseases were: acute leukemias (78), lymphoproliferative disorders (22), myelodysplastic syndromes (15), chronic myeloproliferative diseases (12), multiple myeloma (8) and bone marrow failures (6). Donor was unrelated in 79 cases, and related in 62 (including 23 haplo-identical). Conditioning regimen was: busulphan-based (93), melphalan-based (17), TBI-based (17) and others (14). Progenitors source was PB in 128, and BM in 13. Platelet count, %IPF and absolute IP count (aIPC) from day +1 to the day of stable graft were analyzed. 52.4% patients reached  $\text{plat} \geq 20\ 000/\text{mCL}$  at day +14, 82.1% at day +21 and 86.9% at day +28. Median first day of  $\text{plat} \geq 20\ 000/\text{mCL}$  was day +14 (range: 4–42). Median %IPF was 2.6% (range: 0–15.4), 2.5% (range: 0–28.4) and 3.65% (range: 0–15.3) at days +9, +10 and +11, respectively. Median aIPC was 292/mCL (range: 0–2835), 336 (range: 0–2840) and 504 (range: 0–3660) at days +9, +10 and +11, respectively. Among the time points analyzed, aIPC at day +11 showed the best positive correlation with platelets counts at day +14 ( $R=0.72$ ). Interestingly, patients with lower aIPC at day +11 showed a delayed platelet graft (see Table 1). Contrarily, patients with higher aIPC at day +11 had an earlier platelet graft. Absolute immature platelet count before the graft seems to predict the precocity of the platelet graft for the majority of patients undergoing allo-HSCT. This finding might help physicians for the patient management (anticipation of hospital discharge and so on).

**Disclosure of conflict of interest:** None.

### [P001]

Table 1. Influence of immature platelet count at day +11 on platelet graft.

aIPC at + day 11 (/mcl)	Platetel counts > 20000/mcl		
	At day +14	At day +21	At day +28
<b>N=84</b>			
< 100 (N=5)	0 (0%)	2 (40%)	2 (40%)
< 200 (N=13)	4 (30.8%)	7 (53.8%)	8 (61.5%)
≤ 504 (median of the series) (N=43)	17 (39.5%)	31 (72.1%)	33 (76.7%)
> 504 (median of the series) (N=41)	27 (65.9%)	38 (92.7%)	40 (97.6%)

p < 0.05

### P002

#### Analysis of genetic polymorphism for cardiovascular diseases (CVD) in placental and maternal blood in hypertension and hypercholesterolemia

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Cardiovascular Diseases are the world's leading cause of death representing 30% of the total global mortality. The genetic polymorphism of the 12 CVD genes, especially the ACE: Angiotensin Converting Enzyme gene risky alleles (INS/DEL) which are associated with a high and inappropriate level of ACE can be considered as a genetic model in the development of hypertension and its complications in CVD. We evaluated the mutation impact of the 12 CVD genes in the Lebanese population, based on 40 samples derived from placental blood (PB) and 40 samples derived from peripheral blood of postpartum mothers. Adult females (Age ≤ 35 years) were divided (N=20 per group) into group1 (normotensive, normocholesterolemia: NN), and group 2 (hypertension, hypercholesterolemia: HH). Buffy coat were extracted from the 40 PB. All tests on PB and maternal blood were done by using the Test strip Assay to identify the most relevant genetic variations to estimate the risk for CVD. The presence of a double mutation (INS+/DEL+) related to the ACE gene in the HH group was 75%. The presence of a single mutation (INS-/DEL+) was only associated to the HH by 25%. (INS-/DEL-) was absent in 100% of the PB and NN. Despite the presence of double mutation INS/DEL for CVD in maternal blood, PB was free of this mutation. Therefore, beyond genetic mutations, other factors can play a major role in the occurrence of CVD.

**Disclosure of conflict of interest:** None.

**P003**

**Automated red blood cell depletion in ABO incompatible grafts in the pediatric setting**

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Red blood cell (RBC) depletion by apheresis is employed to reduce the RBC content from ABO major or bidirectional mismatch bone marrow (BM) grafts mainly to avoid severe haemolysis. RBC depletion results in a significant volume reduction (due to both RBC and plasma depletion) and buffy coat concentration 2.3. In pediatric setting, both RBC depletion and volume reduction before transplantation or cryopreservation can avoid fluid overload and renal impairment, especially in low/very low body weight recipients. The aim of this study was to evaluate the quality of the graft and immediate post infusion complications in RBC depleted BM in major and minor ABO mismatch recipients using an automated device.

**Patients and methods:** BM aspirates for transplantation in pediatric setting were processed at our Centre using the Spectra Optia (Terumo BCT) automated device. The initial collection preference was set at level 30 and then was adjusted in order to maintain a haematocrit of 5% (colorgram) in the collection bag. Flow speed was set at 120mL/min for 10 cycles. Mean recipients' body weight was 31 kg (range:11–72). Pre and post procedure BM bag volume, HCT%, mononuclear cells (MNCs) count, (including B and T lymphocytes), CD34+ cell and cell viability were calculated. Moreover, post procedure RBC volume and procedure time were registered. On the patient's side, post infusion complications (renal impairment, fluid overload, fever and haemolytic reactions) and time to engraftment were evaluated. **Results:** A total of 20 RBC depletion procedures were consecutively performed on 20 BM grafts (13 major and 7 minor ABO incompatibility, 16 MUD and 4 related donors). Data about pre and post procedure graft composition are reported in table 1. Mean time to engraftment for PMN was 22.6 days (range:17–34) and for PLT was 33.5 (range: 21–43). Pre and post-procedure cell viability were always >97%. Mean procedure time was 80.6 minutes (range:59–115). No bacterial or fungal contamination was detected. No infusion complications were recorded. One graft failure was observed. Conclusions The Spectra Optia automated system is efficient in RBC depletion of ABO mismatched grafts, permitting an effective volume reduction and an excellent MNCs and CD34+ cell recovery in pediatric setting. Automated RBC depletion may be proposed in low/very low body weight recipients both in ABO major and minor incompatibility setting to minimize graft infusion side effects.

[P003]

	Pre-RBC depletion Mean (range)	Post-RBC depletion Mean (range)	Recovery (%) Mean (range)	Depletion (%) Mean (range)
BM bag volume (ml)	987.4 (417-2100)	134.4 (67-189)	-	88.2 (85.1-98.2)
HCT%	32.9 (25.3-43)	5.2 (2.9-10)	-	-
Bag RBC content (ml)	321.7 (155.1-657.3)	6.9 (3-11.1)	-	97.8 (95.4-98.3)
Total CD34+ (x10 <sup>6</sup> )	176.2 (22.3-412.18)	162.2 (22.2-292.5)	96.8 (70.8-161.1)	-
CD34+/kg (x10 <sup>6</sup> )	6.7 (1.2-25.6)	6.1 (1.3-20.8)	-	-
Total MNCs (x10 <sup>9</sup> )	4.1 (1.15-7.2)	3.8 (2.3-5.4)	82.2 (67.1-95.2)	-
T-Lympho/kg (x10 <sup>6</sup> )	68.7 (29.5-130.1)	65.7 (15.6-125.7)	-	-
B-Lympho/kg (x10 <sup>6</sup> )	19.2 (5.3-45.7)	19.5 (5.25-47.9)	-	-

TABLE 1. Pre and post RBC depletion graft characteristics.

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**Disclosure of conflict of interest:** None.

**P004**

**Building up a hematopoietic stem cell transplantation program in a public hospital in Uruguay: International cooperation and development program**

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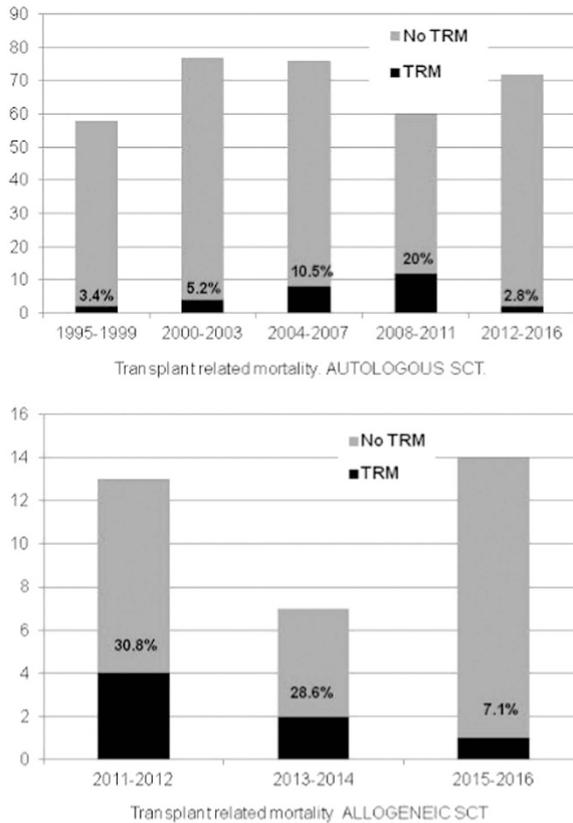
Building up a Stem Cell Transplantation program in an emergent country, in the public setting, with limited economic resources, is not an easy work to do. International cooperation may be essential for the development of the program, in training, technological support and implementation of international guidelines. After 20 years, we show an experience of international cooperation between a highly developed center in France (Institut Paoli Calmettes, Marseille) and the Stem Cell Transplantation Department of Hospital Maciel, a Public Assistance Service in Montevideo, Uruguay. Fourteen persons between doctors and nurses have been trained in France in stem cell collection and processing, patient's clinical handling, nursing, outpatients care and quality management. French missions of experts have been also received in Hospital Maciel every year since 2002 for *in situ* human resources training. In last 5 years we developed a program for optimizing transplant results and reducing transplant related mortality (TRM), based on several measures: improvement of patients selection, applying the Sorror Comorbidity Index; adjustment of conditioning regimen doses, in order to reduce toxicity; development of a program to improve interaction with the Intensive Care Unit; protocolization of the standard proceedings treatments; and initiating a program of quality and safety at the National Institute of Quality of Uruguay INACAL. 456 adult patients have been treated with autologous (ASCT) (347) or allogeneic (alloSCT) (109) SCT, with hematological malignancies. Different modalities of alloSCT have been included progressively, becoming the only center accredited by the national regulation authorities (FNR) to perform unrelated donor SCT and the haploidentical donor SCT. This increased the proportion of allogeneic transplants from the historical 20% until 33% in last 2 years. Regarding patients health coverage, 45% comes from the private assistance system and 55% from the public health system. The major indications are lymphoid malignancies and acute leukemia, for ASCT and alloSCT, respectively, showing the same trend than CIBMTR. Three-year overall survival (OS) for acute myeloid leukemia after alloSCT is 61%. Considering ASCT for diffuse large B cell lymphoma, 3 years OS after autologous SCT is 82% and 52% for chemo sensitive and resistant disease, respectively. Three-years OS after ASCT for Hodgkin disease is 87 and 67% for sensitive and resistant disease, respectively. ASCT in multiple myeloma shows an OS of 69 and 50% at 3 and 5 years, respectively. In TRM, results during the last 5 years (after the described strategy) are shown in Figure 1. The development of the—program of continuous improvement in quality—and the impact of results was locally recognized by two annual prizes from INACAL in 2013 (Bronze) and 2014 (Silver) in the category 'Commitment to Public Service.' A successful

transplant program can be established in a developing country at the public setting, based on international collaboration and interaction with a twin highly developed center. Development of a follow up program considering local aspects is essential in order to enhance the center effect in the results.

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Figure 1 [P004].



**Disclosure of conflict of interest:** None.

#### P005

Previously published

#### P006

##### Correlation between miRNA expression and the level of proangiogenic cytokines in autologous hematopoietic stem cell transplantation

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MicroRNAs play an important role in regulation of angiogenesis and hematopoiesis hence influence hematopoietic stem cells (HSC) homing after transplantation by targeting bone marrow niche microenvironment, especially cytokines. The aim of the study was to evaluate a relationship between the expression of selected miRNAs: miRNA-16, miRNA-146a,

miRNA-223 and the level of proangiogenic cytokines: angiopoietin-1 (ANGPT1), matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) in patients with lymphoproliferative malignancies prior to autologous hematopoietic stem cell transplantation (HSCT) and in early post-transplant period. Twenty-four patients were enrolled to the study (11 F,13 M). The median (Me) age was 57 years. The investigated group consisted of 19 multiple myeloma and 5 lymphoma patients. The plasma samples were collected on 4 time points: before chemotherapy—'BC', on the day of HSCT—'0', 7 days after HSCT—'+7' and 14 days after HSCT—'+14.' The cytokines were evaluated using ELISA method, while miRNA levels were estimated by qPCR method. The Wilcoxon matched-pairs test was used to compare groups of dependent continuous variables: miRNA's Relative Quantification (RQ) levels or cytokines expression at two different time points. Spearman rank correlation coefficient (R) was used to compare independent variables. We observed continuous decline of cytokines and miRNAs level after conditioning treatment. The deepest decrease of expression was marked on '+7' day (Table 1).

[P006]

Table 1. Changes in miRNA and cytokines level before conditioning treatment and after HSCT

miRNA/Cytokines Median	"BC" day	"0" day	" +7" day	" +14" day
		p values as compared to baseline		
miRNA-16[RQ]	Me:1.60	Me:0.94,p=0.01	Me:0.10,p<0.001	Me:0.08,p<0.001
miRNA-146a[RQ]	Me:1.25	Me:0.64,p=0.059	Me:0.07,p<0.001	Me:0.06,p<0.001
miRNA-223[RQ]	Me:1.47	Me:0.54,p=0.059	Me:0.04,p<0.001	Me:0.06,p<0.001
ANGPT-1[pg/mL]	Me:3720	Me:3111.8,p=0.215	Me:906,p<0.001	Me:1234.5,p<0.001
MMP-9[ng/mL]	Me:23	Me:35.5,p=0.918	Me:0,p<0.001	Me:12,p=0.002
VEGF[pg/mL]	Me:80.9	Me:58.05,p=0.055	Me:43.2,p=0.008	Me:37.8,p=0.003

We noticed a positive correlation between miRNA-16, miRNA-146a, miRNA-223 expression on '0' day and ANGPT-1 level: (R=0.47, p=0.022), (R=0.47, p=0.02), (R=0.52, p=0.009) respectively. A similar correlation at this time point was observed in case of MMP-9: (R=0.69, p<0.001), (R=0.53, p=0.008), (R=0.52, p=0.01) respectively. Additionally we found the positive correlation between levels of miRNA-16, miRNA-146a and miRNA-223 assessed on '+7' day and MMP-9 expression: (R=0.43, p=0.04), (R=0.52, p=0.009) and (R=0.51, p=0.01) respectively. MiRNA-146a and miRNA-223 measured on '+14' day after HSCT positively correlated with VEGF concentration (R=0.56, P=0.006), (R=0.51, P=0.01). The expression of miRNAs influencing hematopoiesis, homing and angiogenesis correlates with the level of cytokines regulating neovascularization in critical time points during autologous stem cell transplantation. Our results suggest key role of angiogenesis in regeneration of hematopoiesis controlled by different miRNAs.

**Disclosure of conflict of interest:** None.

#### P007

##### Exclusion of Trypan blue exclusion test for CD34+ cell viability determination

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Trypan blue dye exclusion assay is one of the most commonly used tests for measuring cell viability. Nonviable nucleated cells, due to the loss of the membrane integrity and function, are stained blue, when living cells remain intact. Addition of cryoprotectant, along with cryopreservation and thawing process has impact on cell viability in peripheral blood stem cells (PBSC) product. The aim of our study was to evaluate the benefit of trypan blue exclusion test for measuring viability of

cells in PBSC product. Among the autologous transplanted patients between March 2011 and October 2016, we have selected 170 according to diagnosis, conditioning regimen and number of infused bags of cryopreserved PBSC. This group included 77 females and 93 males with median age of 56 (range: 19–71). Most of them, 105 (61.76%), had multiple myeloma (MM), 41 (24.12%) had non-Hodgkin's lymphoma (NHL) and 24 (14.12%) had Hodgkin's disease (HD). After harvesting, CD34+ cell and leukocyte number in PBSC product were enumerated on flow cytometer and blood cell counter, respectively. PBSC were cryopreserved with 10% dymethyl sulfoxide (DMSO) and cell viability was measured with trypan blue exclusion test before and after adding DMSO, and as well after thawing in water bath on 37°C. As a conditioning regimen for the MM patients, melphalan was used and for the NHL and HD patients we used BEAM regimen. All received one bag of cryopreserved PBSC and pegfilgrastim 6 mg on the first or the second post-transplant day. Time to hematopoietic recovery was measured; for neutrophils  $>0.5 \times 10^9/L$ , leukocytes  $>1 \times 10^9/L$  and platelets  $>20 \times 10^9/L$  with at least 2 days without platelet transfusion. The median number of total leukocytes infused was  $91.88 \times 10^9/L$  (range:  $29.27-284.87 \times 10^9/L$ ) of which CD34+ cells were  $2-24.09 \times 10^6/kg$  of patient's body mass (median  $4.75 \times 10^6/kg$ ). Pre-freezing cell viability before and after adding DMSO was with a median of 100% (74.1–100) and 81, 75% (26.7–100), respectively, and post-thaw viability 57.37% (16.7–100). The average time to engraftment was 9.8 days (6–26) for neutrophils, 10 days (6–27) for leukocytes and 10.8 days (8–22) for platelets. Our results confirmed the known correlation between the number of infused CD34+ cells and engraftment of neutrophils ( $P < 0.0001$ ), leukocytes ( $P < 0.0001$ ) and platelets ( $P = 0.0005$ ). We found inverse correlation between the infused leukocytes and cell viability with DMSO ( $P = 0.0035$ ) and after thawing ( $P = 0.0019$ ). No correlation was found between pre-freezing and post-thaw viability with hematopoietic recovery, and also between the CD34+ number and these viabilities. No differences were found considering patients' age, gender, diagnosis, conditioning regimen or day of applying pegfilgrastim. We can indirectly infer good survival of CD34+ cells and higher sensitivity of other nucleated cells to preparation of PBSC product. Trypan blue exclusion assay, due to its inability to distinguish type of stained cells, is not relevant for CD34+ cells survival determination.

**Disclosure of conflict of interest:** None.

#### P008

**Previously published**

#### P009

**Previously published**

#### P010

**Improved outcomes of unrelated umbilical cord blood transplantation in children with chronic granulomatous disease: results of a single center in China**

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Chronic granulomatous disease (CGD) is a kind of primary immunodeficiency disorder of phagocytic cells which resulting in failure to kill a defined spectrum of bacteria and fungi and in concomitant chronic granulomatous inflammation. Allogeneic hematopoietic stem cell transplantation is the only treatment proved to be potentially curative in CGD. Unrelated umbilical cord blood (UCB) is increasingly used as an alternative to bone marrow. **Methods:** unrelated UCBT was performed 14

consecutive CGD children at our center between 2015 and 2016. Median age was 18.5 months (range: 5–143 months), median body weight was 10.3 kg (range: 8–34 kg). All patients received myeloablative conditioning regimen consisting of busulfan, fludarabine, cytarabine, cyclophosphamide and G-CSF. All patients received tacrolimus as prophylaxis for graft-versus-host disease (GVHD). Median nucleated cells were  $9.2 \times 10^7/kg$  (range:  $4.5-15.9 \times 10^7/kg$ ), and median CD34+ cells were  $3.0 \times 10^5/kg$  (range:  $0.9-7.0 \times 10^5/kg$ ). Median follow-up time was 9.5 months (range: 6–23 months) **Results:** 10 of 14 patients engrafted. Median time to neutrophil engraftment was 30 days, and median time to platelet engraftment was 33.5 days. 13/14 patients were alive, and 10/14 had full donor engraftment. Overall survival rate was 92.8%. Disease-free survival was 71.4%. 2 of 14 patients had grades III–IV acute GVHD. No patients developed chronic GVHD. Only one patient died from multi-organ failure related to adenovirus infection. **Conclusion:** Unrelated UCBT should be considered as potential curative methods in children with CGD. Myeloablative conditioning regimen has improved the engraftments of the UCB.

**Disclosure of conflict of interest:** None.

#### P011

**Influence of muscle mass and visceral fat (evaluated by ultrasound) and of muscular strength on the engraftment time in patients undergoing hematopoietic stem cell transplant (HSCT)**

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Reduced muscular mass and excess visceral fat in patients undergoing HSCT are associated with higher mortality, longer hospitalization, longer use of immunosuppressive drugs, graft-versus-host disease (GVHD) and comorbidities leading to shorter survival time. A recent study of patients undergoing allogeneic HSCT showed that occurrence of enlarged areas of visceral and peripheral fat is inversely associated with the disease-free interval after the transplant. Reduced muscle mass has also been associated with higher prevalence of chronic GVHD and low rates of success following allogeneic HSCT. **Objectives:** To investigate whether amount muscle mass and muscle strength (MS) as well as the amount of visceral fat (VF) of patients undergoing HSCT would influence the duration of the engraftment time (EN). We evaluated 14 HSCT patients ( $\geq 18$  years) at Hospital Israelita Albert Einstein, São Paulo, Brazil, on their first day of hospitalization, before HSCT. The thickness of the right femoral quadriceps muscle (RFQ), measured at 6 cm from the top edge of the patella was measured using ultrasound (US) in B-mode. The dominant upper limb strength of the patients was evaluated by the hand grip test. The VF was measured in the abdominal region, by the thickness of the fat layer between the linea alba and the anterior wall of the aorta. Most patients were women (57%) with a mean age of 50 years ( $\pm 16$  years) and 50% of our patients were elderly ( $\geq 60$  years). The haploidentical (57%) was the predominant HSCT, autologous (36%) and allogeneic (7%). Most patients were overweight, with body mass index (BMI) of  $27 kg/m^2$  ( $\pm 4 kg/m^2$ ). The average time EN was 16 days ( $\pm 6$  days). RFQ was 1.5 cm ( $\pm 0.3$  cm), MS was 31 kgf ( $\pm 7.0$  kgf) and the VF was 5.3 cm ( $\pm 1.4$  cm). Patients with lower RFQ had a longer engraftment time that was statistically significant as the negative correlation between RFQ and EN was  $r_s = 0.8$ ,  $P < 0.05$ , independent of the age and the HSCT type as analyzed by Linear Regression. No significant correlation between VF or MS with EN was found. In this cohort of patients we found that longer engraftment times were significantly correlated to reduced muscle mass but no positive or negative correlation was found with superior limb muscular force or with the amount of visceral fat.

**Disclosure of conflict of interest:** None.

## P012

### Inpatient exercise therapy vs relaxation and mental training in pediatric stem cell transplantation: Results of the RCT BISON

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Hematopoietic stem cell transplantation (HSCT) has been increasingly effective in the treatment of malignant and non-malignant hematopoietic diseases, though it is also associated with a rapid decline of physical capacity and psychosocial well-being. Despite the beneficial effects of exercise have been well established in adult populations, RCT's in the pediatric HSCT are still lacking. The following study was performed to evaluate the physical and psychological impact of an inpatient exercise program for children and adolescents during HSCT. After informed consent all participants ( $n=70$ ) were randomized into an exercise intervention (IG) or a non-exercise control group (CG). While the IG included a combined resistance, endurance and flexibility training, the CG performed a mental training and relaxation exercises. During inpatient treatment, the daily sessions last about 40–60 min for each group. Dependent variables included maximum isometric quadriceps strength relative to body weight (QS; strain gauge force transducer), hand grip strength (HGS; JAMAR dynamometer), the distance walked in 6 min (6MWD; 6-min walk test), quality of life (QoL; age-corresponding KINDL-R questionnaire) and medical parameters. Assessments were prior to admission to the hospital for HSCT (T0) and the day of discharge (T1). Between 2011 and 2014, 57 patients (IG  $n=28$ ,  $10.9 \pm 3.5$  years vs CG  $n=29$ ,  $10.8 \pm 3.8$  years) completed the study successfully. During the hospitalization phase ( $41 \pm 12.9$  days) patients attended in  $3.1 \pm 0.6$  or  $2.7 \pm 1.1$  sessions ( $156.3 \pm 42.4$  or  $140.8 \pm 69.6$  min) per week. First results indicate that the IG was discharged 3 days earlier than the CG. In 6MWD, HGS and QS significant decreases ( $P < 0.05$ ) were observed in the CG, while the IG-values did not differ between T0 and T1 (6MWD:  $-18.1 \pm 19.4$  vs  $-1.2 \pm 19.5\%$ ; HGF:  $-10.9 \pm 20\%$  vs  $-3.6 \pm 16\%$ , QS:  $-7.9 \pm 38.8\%$  vs  $3.8 \pm 37.4\%$ ). For QoL a significant reduction in both groups were analyzed (Total score  $P < 0.05$ ). Further data analysis revealed significant differences between groups ( $P < 0.05$ ) concerning the change in 6MWD, HGS and Physical wellbeing (QoL) from T0 to T1. A supervised and individually adapted exercise therapy should be considered as part of the rehabilitation program during the acute treatment phase in children and adolescents. Our results indicate that a moderate to vigorous program is not only feasible, but effective in preventing the transplant-related decline of physical performance. However, further studies are needed to confirm these findings in pediatric populations.

**Disclosure of conflict of interest:** None.

## P013

### Lymphocyte recovery and long-term outcome in allogeneic hematopoietic stem cell transplantation

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**Introduction:** lymphocytes are the cells responsible for the cellular and humoral immunity and, consequently, critical for hematological patients. The aim of this study was to analyze the eventual connexion between lymphocyte recovery and survival (SRV) after allogeneic hematopoietic stem cell

transplantation (allo-HSCT). **Patients and methods:** we retrospectively analyzed data from 223 consecutive patients who underwent allo-transplants in our Unit. In total, 126 patients were male (56.5%) and 97 female (43.5%). Median age was 53 years old (range: 8–72). Baseline disease was: acute leukemia (56.9%), lymphoma (11.2%), myelodysplastic syndrome (10.3%), chronic myelogenous leukemia (8.9%), multiple myeloma (4%), aplastic anemia (3.58%), chronic lymphocytic leukemia (3.13%) and others (1.79%). 55.1% of allo-HSCTs were from an unrelated donor, and 44.9% from a family donor (25% of them haplo-identical). The SC source was PBSC in 89.6%, and BM in 10.4%. A variety of conditioning regimens were employed, including: busulphan-based (69.5%), melphalan based (10.4%), TBI-based (9.86%) and others (9.86%). Evolution of absolute lymphocyte counts (ALC) and subpopulations during the first year after allo-HSCT were analyzed. **Results:** as shown in Table 1, ALC decreased abruptly during conditioning therapy and recovered up to baseline at days +30 and +100; at day +365 median ALC had clearly improved compared with admission values. Median CD4+ cells were lower than 500/mcL in two thirds of pts at day +100 and in only one third at day +365. As shown in Table 2, we found a significant link between ALC at day +30 and SRV, as well as between CD4+ cells at day +100 and SRV. In our series, immunity recovery was a late event for the majority of patients undergoing allo-HSCT. In addition, in our experience, the precocity and quality of the ALC and CD4+ recovery was clearly linked with long-term survival.

**Disclosure of conflict of interest:** None.

## P014

### Mesenchymal stem cell chimerism following HSCT

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Although there is experimental evidence suggesting the presence of a common mesoderm cell as origin of both hematopoietic (HSC) and mesenchymal progenitor cells (MSC) in an animal model, it is still controversial if durable engraftment of native donor-derived MSCs without *ex vivo* treatment can occur in the recipient of allogeneic HSCT. To assess the presence of donor-derived MSC following HSCT. Between July 2015 and July 2016, a total of 33 recipients of HSCT were analyzed for HSC and MSC chimerism. Eighteen patients received BM grafts (54%), 11 patients had peripheral blood as stem cell rescue (33%) and finally 3 patients had a cord blood transplantation (9%). Patients received myeloablative (91%) or reduced intensity conditioning (9%) for malignant (91%) or nonmalignant disease (9%). BM aspirate cells were plated and expanded in  $\alpha$ -MEM with 10% Human Platelet Lysate at  $10^6$  cells/cm<sup>2</sup>. After 5–7 days, nonadherent cells were removed, while the adherent cells were expanded until they reached confluence. After 2 weeks we quantified MSC precursors as colony forming unit fibroblast (CFU-F). Finally the amplified sequences were resolved by capillary electrophoresis (3500 Ruo Genetic Analyzer, Applied Biosystems) and analyzed by comparing genotypes of BMT recipell detachment, nuclear DNA was extracted (DNeasy Blood and Tissue kit—Applied Biosystems) and specific polymorphic tandemly repeated regions (STRs) were amplified by means of the polymerase chain reaction (PCR) following the specific manufacturers' instructions. (AmpFSTR Identifile kit, Applied Biosystems following HSCT (HSC and MSC) to those of donors. We cultured 54 whole BM aspirates from patients following HSCT with a median time of 244 day (range: 41–1606). CFU-F/ $1 \times 10^6$  growth was observed in a majority of BM

samples (37 samples out of 54, 68.5%), enough MSCs to perform the chimerism analysis. All patients showed HSC engraftment while no patients had evidence of donor-derived mesenchymal cell engraftment even when BM aspirate was taken after several years from HSCT. This study shows that MSC after HSCT remain of recipient origin even when BM was analyzed years after HSCT. We do not observe differences after myeloablative or reduced intensity conditioning or regard to underlying disease. The role and the content of MSC in the HSCT graft remains to be established.

**Disclosure of conflict of interest:** None.

## P015

### Morphine consumption during hematopoietic stem cell transplantation strongly correlates with systemic inflammation in pediatric patients

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The great majority of children undergoing myeloablative hematopoietic stem cell transplantation (HSCT) experiences pain and need for opioid analgesia. Nevertheless, the factors influencing the inter-individual difference in pain perceived and the need for opioid analgesics still remain unknown, limiting the possibility of a more individualized pain management. Accordingly, the aim of this study was to investigate if opioid consumption in children undergoing HSCT is associated with measures of gastro-intestinal toxicity (that is, plasma citrulline) and systemic inflammation (plasma IL-6 and CRP), as well as with patient- and transplant-related variables. During 2010–2012 we included 39 children (1–16 years) with malignant ( $n=23$ ) and non-malignant ( $n=16$ ) diseases transplanted with siblings ( $n=7$ ) or unrelated donors ( $n=32$ ) after myeloablative conditioning with ( $n=11$ ) or without ( $n=28$ ) TBI at the National HSCT center in Denmark. Plasma citrulline levels were measured before conditioning and on day +7 and +21 after HSCT by UPLC. Plasma IL-6 was measured at day +7 by Cytometric Bead Array kit. Plasma CRP level was measured daily from day 0 to day +21 by Modular P module. The daily doses of opioids administered intravenously, orally and rectally from day 0 to day +21 were registered retrospectively from the patients' records and converted into intravenous morphine equivalents (ME). 38 patients (97.4%) received opioid analgesics: 5 (12.8%) only orally, 33 (84.6%) intravenously including Patient/Nurse-Controlled Analgesia pumps (in 23 patients, 59%) and 1 rectally plus intravenously (2.6%). The total daily median ME consumption at day 0 was 0.00 mg/kg (IQR 0.00–0.02), which subsequently increased to reach a plateau between day +10 (0.36 mg/kg, IQR 0.18–0.49) and +14 (0.33 mg/kg, IQR 0.15–0.43), and then decreased to 0.02 mg/kg (IQR 0.00–0.13) at day +21. The patients' maximum ME dose was not significantly associated with diagnosis (malignant/benign disease), use of TBI, type of donor (sibling/unrelated), occurrence of GVHD or recipient age. Median citrulline levels decreased from baseline (10  $\mu\text{mol/L}$ , IQR 5–17) to reach a nadir at day +7 (0  $\mu\text{mol/L}$ , IQR 0–7) and then recovered to 8.25  $\mu\text{mol/L}$  (IQR 3.44–12.03) at day +21. Median IL-6 level was increased at day +7 (20 pg/mL, IQR 9–36) compared to healthy controls (<5 pg/mL). Median CRP levels increased from 14 mg/L (IQR 4–26) at day 0 to peak at around day +9 mg/L (16, IQR 6–41) and then decreased to normal levels at day +21 (4.55 mg/L, IQR 1.10–14.68). Correlations between CRP levels and ME consumption were calculated for each day yielding significantly positive correlations on most days with significant  $p$  values ranging from 0.66 ( $P<0.001$ ) at day +4 to 0.35 ( $P=0.041$ ) at day +21. Similarly, IL-6 was correlated with M.E.

at day +7 ( $\rho=0.55$ ,  $P=0.002$ ). In contrast, citrulline levels did not correlate with ME at any time point. These results suggest that the need for opioid analgesics in children receiving HSCT may be influenced by the degree of chemotherapy-induced systemic inflammation, rather than by the extent of enterocyte damage. These findings contribute to the understanding of the mechanisms behind transplant-related pain and may be of importance for developing better pain management strategies.

**Disclosure of conflict of interest:** None.

## P016

### The prevalence of human pegivirus in recipients of allogeneic hematopoietic stem cell

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Human pegivirus (HPgV; previously named as GB virus C/Hepatitis G virus) was discovered more than 20 years ago. It is an RNA virus referred within the genus Pegivirus of the family Flaviviridae. HPgV RNA is found in liver, spleen, bone marrow and peripheral blood mononuclear cell, including T- and B-lymphocytes, NK-cells and monocytes. Despite of the fact that it is a molecular structure, mechanism of replication and transmission routes are very well understood but the clinical significance of HPgV is still not determined. Recipients of allogeneic hematopoietic stem cell have a high risk infection of HPgV. It is known, that HPgV is a non-pathogenic virus, however, it may play a role in immunocompromised individuals. To investigate the frequency of occurrence of HPgV and its clinical significance in recipients of allogeneic hematopoietic stem cell. Blood samples were obtained from 101 patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT): ALL  $n=21$ , AML  $n=53$ , MPN  $n=7$ , CLL  $n=1$ , MM  $n=1$ , LPD  $n=4$ , AA  $n=6$ , MDS  $n=8$ . A median of age was 33 years (19–64 years). Forty five patients were males and 56 patients were females. Conditioning regimen was RIC in 75 cases, MAC in 26. Bone marrow as a graft source was used in 68, PBSC—33. All patients received multiple transfusions of blood components at the previous stages of treatment. HPgV RNA had been assayed by polymerase chain reaction real time (RT-PCR) on plasma samples before started pre-transplantation conditioning. Despite the diagnosis incidence of HPgV was high 53.5% (RNA-HPgV was positively in 54 patients). Patients with piercings and tattoos had incidence of HPgV in 64% that was not statistically significant ( $P>0.5$ ). HPgV is known as non-hepatotropic virus. In our study there was also no statistical reliability of specific changes in liver function test such as elevating the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin due to the RNA-HPgV. Liver enlargement was also not statistically significant according to ultrasound scan results in patients infected with HPgV. We also analyzed the co-infection with hepatitis B and C virus. Results are presented in Table 1. Co-infection was not statistically significant. However, only one patient with hepatitis C was coinfecting HPgV. Leukocytes recovery median was 22 days (14–55). Thrombocytes recovery median was 23 (11–82). The presence of RNA-HPgV did not affect the recovery of peripheral blood cells in patients after allo-HSCT. According to our study the frequency of HPgV infection in recipients of allogeneic bone marrow was quite high (53.5%), and it did not depend on the presence of any other hepatotropic viruses. Clinical significance of HPgV infection in recipients of allogeneic hematopoietic stem cell has not been revealed, it is possible due to the short follow-up. It needs further clinical research.

[P016]

Hepatitis B and C markers	RNA: HPgV negative	RNA: HPgV positive
HBsAg	2	0
anti-Hbcor	12	8
anti-Hbe	3	2
DNA-HBV	3	0
anti-HCV	1	1
RNA-HCV	0	1

**Disclosure of conflict of interest:** None.

**P017**

**Quantification of CD31+ recent thymic emigrants and T cell receptor excision circles (TRECs) in umbilical cord blood transplanted patients**

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Reconstitution of T lymphocytes is a limiting factor in the regeneration of an effective immune system in adult patients following hematopoietic stem cell transplantation. CD31 (PECAM-1) is a transmembrane glycoprotein expressed on naive T-cells that have recently emigrated from the thymus into the periphery. In peripheral blood, CD31<sup>+</sup> T lymphocytes also contain high numbers of T-cell receptor excision circles (TRECs); excision loops of DNA excised during T-cell receptor gene rearrangement during T cell maturation within the thymus (1–3). However, quantification and correlation of CD31 and TREC has not been formally investigated in patients following umbilical cord blood (CB) transplantation. Quantification of CD31 and TRECs post CB transplant will provide an insight into the immune reconstitution of T cells from the thymus. We therefore sought to measure CD31 and TRECs in patients after CB transplant and assess whether these markers provided evidence of thymic recovery. We followed 67 adult patients (median age 52.9 years) who underwent CB transplant in the UK. Patient samples were collected 28, 60, 100, 180, 365 and 730 days post transplant. Using flow cytometry, we determined absolute counts of CD4+CD31+CD45RA+ and CD8+CD31+CD45RA+, and quantified the copy numbers of TREC genes in peripheral blood mononuclear cells (PBMCs) via real time PCR. **Results:** At the six time points, the number of samples collected were the following: 44, 39, 37, 22, 24 and 14. In all of the samples, the overall median number of CD4+CD31+CD45RA+ was 29 cells/ $\mu$ L (range: 0–827 cells/ $\mu$ L). The median level of CD4+CD31+CD45RA+ cells increases from 16 to 50 cells/ $\mu$ L from day 28 to day 365. Absolute counts of CD4+CD31+CD45RA+ at all of the six time points is 10-fold lower compared to healthy controls (median: 279 cells/ $\mu$ L, range: 105–523 cells/ $\mu$ L). The overall median number of CD8+CD31+CD45RA+ cells is 30 cells/ $\mu$ L (range: 0–2222 cells/ $\mu$ L). There is an increase in the median number of CD8+CD31+CD45RA+ cells between days 28 and 720 post-transplant from 2 to 92 cells/ $\mu$ L. However, the absolute median counts of CD8+CD31+CD45RA+ cells in patients are twofold lower, 2 years post transplant, compared to healthy controls (median: 252 cells/ $\mu$ L, range: 133–503 cells/ $\mu$ L). In the majority of the patient samples throughout all time points the TREC gene copy numbers were undetected ( $n=132$ ). In a few patient samples ( $n=9$ ) TREC gene copy numbers were quantified but with this limited sample size no correlations can be made between the absolute counts and TREC gene copy numbers. Our data suggests that cord blood transplant patients within the UK have reduced levels of CD4+CD31

+CD45RA+ and CD8+CD31+CD45RA+ thymic emigrants for up to 2 years post transplant. Moreover, TREC gene copies were not detected in patient samples; supporting the finding that recovery of naive T cells is reduced following CB transplant.

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**Disclosure of conflict of interest:** None.

**P018**

**Remission after allogeneic hematopoietic stem cell transplantation in a patient with LPS-responsive beige-like anchor (LRBA) gene mutation**

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**Introduction:** Common variable immunodeficiency (CVID) is a highly heterogeneous group of primary immunodeficiency characterized by defective antibody production, recurrent infections, lymphoproliferation and autoimmunity. Autosomal recessive mutations in LRBA, encoding LPS-responsive beige-like anchor protein were first described as a cause of CVID-like disease in 2012. Although HSCT is accepted as a standard treatment modality for long-term resolution of severe primary immunodeficiencies, its role is less established in patients with LRBA deficiency. Patients and methods: Whole exome sequencing of patient's genomic DNA obtained prior to the HSCT revealed a homozygous deletion in LRBA (c.5527delT.p. C1843fs). Immunological analyses including serum immunoglobulin levels, flow cytometry analyses of lymphocyte subsets, cytotoxicity/proliferation assays, vaccine responses were studied at several time points throughout the disease course, prior to and after HSCT. A 14-year-old boy, born to consanguineous healthy parents of Turkish origin became symptomatic at the age of 6 months. He hospitalized several times due to recurrent pulmonary infections. He developed pancytopenia, lymphadenopathy, hepatosplenomegaly and autoimmunity (autoimmune hemolytic anemia and thyroiditis) with low serum immunoglobulin levels at the age of 4. As a result, he received several courses of steroid and prophylactic immunoglobulin and wide-spectrum antibiotics. Over time he manifested growth failure and diagnosed with IBD-like colitis. Due to the cumulating severe CVID-related complications, a HSCT was performed at the age of 14 years with the bone marrow stem cells from his HLA identical brother after a conditioning regimen including fludarabine, busulfan and ATG. Severe intractable colitis with hypoalbuminemia continued till the engraftment despite vigorous fluid-electrolyte replacement therapy and accompanied with severe episodes of acute gastrointestinal bleeding. After the achievement of full donor chimerism, diarrhea episodes resolved. He received three doses of abatasept because of persistent cytopenia thinking about unresolved immune dysregulation. He is in complete remission at 1-year post-HSCT with no signs of graft versus host disease. Allogeneic HSCT should be considered in patients with LRBA deficiency prior to the development of disease-related severe cumulative manifestations.

**Disclosure of conflict of interest:** None.

**P019****Second haploidentical stem cell transplantation as a salvage therapy for children with acute leukemia relapsed after first allo-HSCT**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for acute leukemia in children. However, post-HSCT relapse remains a major cause of treatment failure with relapse rates ranging from 20% to 60%. Optimal treatment of relapse after HSCT remains unclear. One of the possible options might be second transplantation from another donor. Retrospectively we analyzed the outcomes of 21 children receiving in 2008–2015 the second haploidentical HSCTs (haplo-HSCT) performed for post allo-HSCT relapse of acute leukemia (6 AML, 14 ALL, 1 mixed lineage leukemia). Median age at the time of the first HSCT was 5 y.o. (13 m.o.–17 y.o.), median age at second HSCT was 6 y.o. (22 m.o.–17 y.o.). The first allo-HSCT was performed from matched unrelated ( $n=7$ ), matched related ( $n=7$ ), haploidentical ( $n=6$ ), syngeneic ( $n=1$ ). Condition regimen was MAC in 14 pts (67%) and RIC in 7 pts (33%). The source of HSCT was BM in 19 pts (90%) and PBSC in 2 pts (10%). Median time from first to second allo-HSCT was 9 months (34 days–28 months). In the case of first haplo-HSCT, the second HSCT was performed from another haploidentical donor. Cytoablative therapy prior to second HSCT performed in 19 pts (90%). There was no response in 16 pts (84%), 1 pt (5%) achieved complete remission and 2 pts (10%) entering aplasia prior to allo-HSCT. The conditioning regimen for the second HSCT was MAC 2 pts (10%), RIC 17 pts (80%), reduced toxicity regimen in 2 pts (10%). The transplant source was G-CSF primed unmanipulated BM in 20 pts (95%), PBSC in 1 pts (5%) with median CD34 + dose  $8.65 \times 10^6/\text{kg}$  b.w (range: 2.5–14.8). Nineteen of 21 patients achieved engraftment, the median time to neutrophil engraftment was 20 days (12–34 days). At the time of analysis, 10 pts were alive with a median follow-up of 2.3 years (0.8–8.3 years). Clinical remission was achieved in 19 pts (90%). The graft failure was observed in two pts (10%). Two-year overall survival (OS) was 47.6%. Seven pts (36%) developed a relapse. Most frequent transplant complications was aGVHD, observed in 14 pts (73%): stage I—three pts, stage II—three pts, stage III—six pts and stage IV—two pts; moderate cGVHD were seen in 12 pts (63%). Causes of death were relapse or progression of disease in seven pts (33%), TRM in four pts (21%) was presented by toxicity (one pts) and acute GVHD (three pts). Patients with cGVHD had significantly better OS, than cGVHD-free (70.1% vs 14.3%;  $P=0.014$ ). Our data showed that the second haplo-HSCT is an acceptable choice for treatment children with acute leukemia relapsed after first allo-HSCT. Further trials are needed to define the role of the second haplo-HSCT including combination with additional treatment after transplantation.

**Disclosure of conflict of interest:** None.

**P020****Successful unrelated umbilical cord blood transplantation with reduce intensity conditioning for very early onset inflammatory bowel disease: an innovative report from China**

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Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract. Very early onset IBD (VEO-IBD) represents those severe children with disease onset occurring

before 6-years-old. Interleukin-10 receptors (IL-10RA, IL-10RB) mutation are considered to be one of the very important genes for VEO-IBD. Currently variant treatment, such as steroid medication, immunosuppressive agents and biological agents could not get complete remission. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was reported to induce remission in those with VEO-IBD. We performed unrelated umbilical cord blood transplantation (UCBT) in five consecutive children with VEO-IBD due to IL-10 receptor mutation between 2015 and 2016. Median age of five children was 15 months (range: 6–46 months), and median body weight was 7 kg (range: 3.2–9.5 kg). All patients received reduced intensity conditioning (RIC) regimen consisting of busulfan, fludarabine and cytarabine. Prophylaxis for graft-versus-host disease (GVHD) was tacrolimus. Most patients (80%) received a 1 or 2 HLA alleles-mismatched cord unit. Median nucleated cells of the cord blood were  $14.3 \times 10^7/\text{kg}$  (range:  $11.2$ – $51.5 \times 10^7/\text{kg}$ ), and median CD34+ cells were  $4.5 \times 10^5/\text{kg}$  (range:  $3.6$ – $14.9 \times 10^5/\text{kg}$ ). Median follow-up time was 10 months (range: 6–24 months). All patients engrafted, median time of neutrophil engraftment was 22 days, and median time of platelet engraftment was 27 days. Four of five patients were alive with continuous donor engraftment, and achieved complete clinical remissions. Colonoscopy at 6 months after transplantation in two children revealed the mucosa healing. Two children had grade III acute graft-versus-host disease (GVHD). One child developed severe chronic GVHD of both lungs and died of ARDS at 6 months after transplantation. It is the first clinical trial that unrelated UCBT was performed in VEO-IBD children in China. Our data should unrelated UCBT with RIC should be considered as a potentially curative therapeutic option in children with VEO-IBD.

**Disclosure of conflict of interest:** None.

**P021****TCR $\alpha\beta$  and CD19-depletion in hematopoietic stem cells transplantation from matched unrelated and haploidentical donors in pediatric patients with refractory acute myeloblastic leukemia**

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Patients with refractory primary induction failure and resistant relapse are poor candidates for hematopoietic stem cell transplantation (HSCT). Additional attempts at remission induction with various combinations of chemotherapy will unlikely improve the outcome and will contribute to excess toxicity. A major goal of SCT has been to develop strategies to reduce the risk of GVHD while maintaining or enhancing GvL. TCR $\alpha\beta$ +CD19+lymphocytes depletion is a technology of graft manipulation with a potential to increase GvL effect and improve GvHD control and immune reconstitution in this group of patients. A total of 31 pts with refractory AML (primary induction failure ( $n=10$ ), refractory relapse ( $n=21$ )), 17 female/14 male, median age 9.7 years (1.4–18), underwent allogeneic SCT between May 2012 and August 2016, median FU 1.5 years (0.3–3.8). 26 pts were transplanted from haploidentical donors and 5 from MUD. All pts had active disease (AD) at the moment of SCT and received treosulfan-based high-intensity conditioning regimen. Three regimens of GvHD prophylaxis were used. Regimen 1 ( $n=7$ ): ATGAM 50 mg/kg with ( $n=5$ ) or without ( $n=2$ ) post-transplant Tacro/MTX; regimen 2 ( $n=9$ ): thymoglobulin 5 mg/kg, rituximab 200 mg/m<sup>2</sup> and post-transplant bortezomib on day+2,+5 ( $n=9$ ); regimen 3 ( $n=15$ ): tocilizumab 8 mg/kg on day-1 and

post-transplant bortezomib ( $n=15$ ), 4 pts receive additional abatacept 10 mg/kg on day+2, +7, +14, +28. TCR $\alpha\beta$ + /CD19 +-depletion of SCT with CliniMACS technology was implemented in all cases. The median dose of infused CD34+ cells was  $8 \times 10^6$ /kg (range: 4.2–17), TCR $\alpha$ /b-14  $\times 10^3$ /kg (range: 2–52). All engrafted pts received additional post-transplant courses of low-dose chemotherapy, including hypomethylating agents and DLI. Primary engraftment was achieved in 27 of 31 pts (three pts had disease progression, one died at the moment of engraftment), the median time to neutrophil and platelet recovery was 12 days (10–25). Early mortality within 100 days was 3.2% (One pt with AML had acute lung injury after engraftment on day +14), 1.5-years pTRM–6.7% (95%CI: 1.7–25). There were no allergic or infusion-related adverse events associated with tocilizumab or abatacept. CI of GvHD grades II–IV and III–IV was 25.8% (95% CI:14–47), and 9.7% (95%CI:3.3–28), respectively. CI of cGvHD was 23% (95% CI:12–42). CI of acute GvHD was lower in a group with prophylaxis regimen without serotherapy: 20% (95% CI:2–28) vs 31.3% (95% CI:7–54) in ATG group. No correlation between graft composition, donor type with the incidence of aGvHD and cGvHD was noted at 1.5 years pPFS (event=death or relapse or progression) was 37% (95% CI:19–54), 1.5-years pOS—48% (95% CI:30–67). Median time of FU for survivors is 1.5 years (range: 0.3–4). We confirm that the depletion of TCR $\alpha\beta$  +/CD19+lymphocytes from the graft ensures high engraftment rate and low transplant-related mortality in pediatric pts with refractory AML. We suggest that tocilizumab and abatacept can be safely administered to children with acute leukemia in the context of treosulfan-based conditioning regimens. Long-term follow-up will demonstrate if the GvHD prophylaxis without serotherapy and combined administration of tocilizumab, abatacept and bortezomib post-TCR $\alpha\beta$ + /CD19 +depleted grafting will improve GvL effects without extensive GvHD-related morbidity and mortality in pts with refractory AML.

**Disclosure of conflict of interest:** None.

## P022

### The JACIE experience at the university hospital of Amiens

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The JACIE (Joint Accreditation Committee of ISCT and EBMT) accreditation aims to improve the management of patients benefiting from autologous or allogeneic hematopoietic stem cell (HSC) transplants. Usually, candidates' centers for JACIE accreditation have already existing clinical activity when they have willingness to comply with JACIE standards. Here, we present our new experience in the implementation of JACIE quality process, at the same time as allograft clinical activity. Implementing process Autograft clinical activity existed at Amiens Hospital, since 1991, but in lack of center cellular therapy laboratory and HSC collection that were outsourced. The collection activity for autologous transplant was set up in 2009, the cellular therapy laboratory in November 2011 and then the allogeneic transplant was started in July 2012. As early as March 2011, we set up a steering committee with hematological clinicians, managers of each sector, a transplant coordinator nurse, the head of the processing laboratory and a part-time quality engineer recruited part-time. Each actor had to become familiar with standards to obtain information from accredited centers in order to evaluate objectives and their prioritization. Steering committee decided on deadlines and established a roadmap including the following: The list of JACIE required standard operating procedures and their writing; assignment of the tasks for each actors in order to evaluate, writing and approval each document; organization of documents diffusion; information to all staff on the approach;

creation of feedback committee for adverse events management; establishment of morbidity and mortality review; formalization of initial and continuing training for medical and paramedical staff; and organization of cross audits with external teams. At the same time, we assessed requirements for starting activity: training for medical and paramedical staff; training for the transplant coordinator nurse; circuits for taking care of donors; the organization of the in-patient department; the organization of follow-up of post-transplant patients; and authorizations of the national regulation agencies for processing facility on manipulations and cellular qualifications and for regulatory collection and transplant. Allogeneic transplant clinical activity started in July 2012. Accreditations JACIE visit occurred on 8 and 9 June 2015 and our center has been officially accredited since 16 March 2016. Neither quality approach, nor clinical activities were easy to implement. Medical and paramedical staff had to get acquainted with a new organization and restrictions. Despite difficulties, implementing JACIE quality process, concomitantly with allograft activity allowed to create a true team dynamics with a common reflection on the means to be implemented. Moreover, quality approach has assured us best ensure to care graft patients. The result is true satisfaction, which be credited to all.

**Disclosure of conflict of interest:** None.

## P023

### Previously published

## P024

### Three-dimensional co-culture of peripheral blood monocytes supports and expands functional hematopoietic stem/progenitor cell without immobilization

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Very low numbers of circulating hematopoietic stem/progenitor cells (CHSPCs) are found in normal human peripheral blood (PB) without mobilization. Here, we developed a three dimension co-culture system to seize and expansion CHSPCs from PB monocytes without mobilization. Flow cytometry analysis was carried out to identify CHSPC phenotypes. Multi-potential properties of CHSPCs were determined using colony-forming unit assay in methylcellulose and reconstitution ability in the compromised animals. The critical regulation mechanism underlying CHSPCs was identified with transcriptome analysis based on next-generation sequencing technology at total or single cell levels. Loose cobble stone colonies (LCs), round or vessel-like compact colonies (RCCs or VCCs) were presented in three dimension co-culture system after about 2 weeks. The colonies lasted for at least six passages with no obvious apoptosis sign, and expanded more than  $\sim 10^3$ -fold during the period. We studied the niche-mediated regulation mechanism of CHSPC fate at molecular level compared to the conventional method of two dimension culture. Furthermore, CHSPCs were capable of forming all types of hematopoietic colonies, including CFU-GEMM, and especially held short term engraftment capacity for compromised NOGs by radiotherapy. Transcriptome analysis by DeepSAGE identified 167 genes significantly associated with regulating the function of CHSPCs. Figure 1: the cellular morphology in three dimension culture system for peripheral blood monocytes without mobilization during the culture for 2–3 weeks. (A–C) round or vessel-like compact colonies. (D) Loose cobble stone colonies. Figure 2: Kinetic expression of CD34+ (A), CD90+ (B), CD309+ (C), CD43+ (D), CD117+ (E), and CD15+ (F) during the three dimension culture of peripheral blood cells without mobilization using flow cytometry analysis.

[P024]

Figure 1

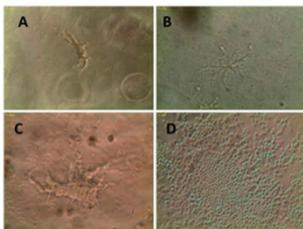


Figure 2

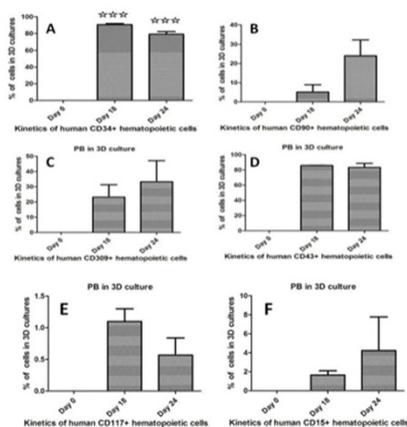


Figure 3

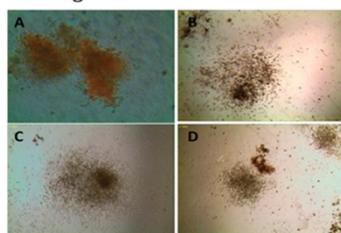


Figure 3: hematopoietic CFU formation assay for the potential of CHSPCs. (A) Burst-forming units of erythroid lineages (BFU-E). (B) CFU-granulocytes and macrophages (CFU-GM). (C) CFU-granulocytes (CFU-G). (D) CFU-granulocytes, erythroid cells, macrophages and megakaryocytes (CFU-GEMM). Figure 4: short transplantable potential analysis of CHSPCs. Figure 5: (A) static of differentially expressed genes between three- and two-dimensional culture systems for peripheral blood cells. (B) GO functional analysis classifies those genes by biological process, cellular component and molecular function. (C) The significant differences between the molecular phenotypes of three- and two-dimension CHSPCs indicating that CHSPCs from three dimension culture hold stem properties. Our system may provide a more ideal and balanced approach which not only seizes circulating CHSPCs, promotes self-renewal and expansion of CHSPCs, but also holds phenotypic and functional attributes of CHSPCs. 6.

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**Disclosure of conflict of interest:** None.

Figure 4

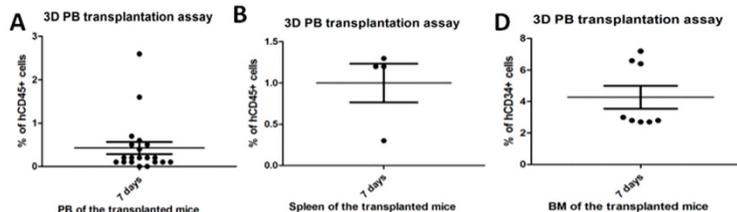
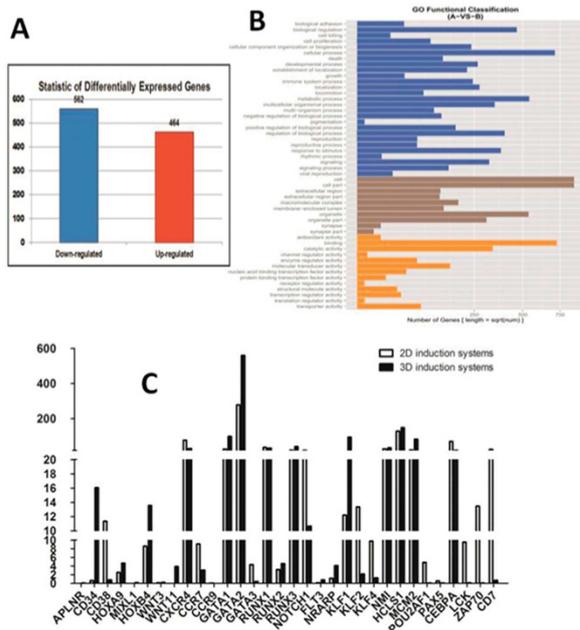


Figure 5



**P025**

**To wash or not to wash? Comparison of neutrophil and platelet engraftment after infusion of cryopreserved autologous stem cells before and after the implementation of bedside thawing**

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Cryopreserved autologous peripheral blood stem cell (PBSC) grafts are widely used after high-dose chemotherapy in the treatment of patients with myeloma or lymphoma. Prior to infusion, cryopreserved grafts can be thawed at the bedside, or thawed and washed at the cell therapy laboratory. At our institution the practice of routine washing of stem cell grafts in the laboratory was discontinued in April 2012 and bedside thawing implemented instead. This was done to minimize the time thawed cells are exposed to toxic DMSO. This study was performed at a single center, at Landspítali—The National University Hospital of Iceland, which is the only transplant center in Iceland. Autologous PBSC transplants have been performed in Iceland since 2004. The study compares outcome for two groups of patients, who received either; (a) thawed and washed autologous PBSC cell grafts from January 2008 to

April 2012, or (b) autologous PBSC grafts thawed at the bedside from April 2012 to November 2016. The following outcomes were compared; days to neutrophil engraftment (absolute neutrophil count (ANC) >0.5 per  $\mu\text{L}$ ), and platelet engraftment (20 and 50  $\times 10^9/\text{L}$ ). Data on mean CD34+ cell content/kg of the infused grafts, measured prior to cryopreservation, were also compared. All patients have received premedication with solucortef, clemastine and ondansetron prior to infusion of the graft. From January 2008 to April 2012 a total of 84 patients received thawed and washed autologous PBSC grafts, and between April 2012 and November 2016 83 patients received autologous PBSC grafts thawed at the bedside. Majority of the patients were diagnosed with either multiple myeloma or related disorders ( $n=86$ ) or lymphoma ( $n=67$ ) whereas the remaining patients ( $n=14$ ) had miscellaneous diagnoses. Days to engraftment and the dose of CD34+ cells infused are compared in Table 1. There was no significant difference in the mean CD34 content of infused autologous stem cells in the two groups (6.9 vs 6.5  $\times 10^6$  CD34+cells/kg,  $P=0.41$ ). There was also no difference in the mean number of days to engraftment of neutrophils (12.8 vs 13.3 days,  $P=0.14$ ), platelets at 20 days (19.2 vs 18.1 days,  $P=0.64$ ) or platelets at 50 days (33.1 vs 28.1 days,  $P=0.62$ ) after transplant. One hundred day mortality was comparable in the two groups or 2.4%. Additional data on transfusion requirements, infections and use of Granulocyte-Colony Stimulating Factor will be presented.

[P025]

Table 1

Thawing procedure	#CD34+cells/kg infused mean $\pm$ SD (range)	Days to engraftment; mean $\pm$ SD (range)		
		ANC>0.5	Plt>20 $\times 10^9/\text{L}$	Plt>50 $\times 10^9/\text{L}$
Cells washed (n=84)	6.9 $\pm$ 3.0 (3.0–19.5)	12.8 $\pm$ 2.2 (9–20)	19.2 $\pm$ 17.1 (0–138)	33.1 $\pm$ 31.6 (11–243)
Bedside thawing (n=83)	6.5 $\pm$ 3.2 (3.0–23.6)	13.3 $\pm$ 2.6 (8–20)	18.1 $\pm$ 11.6 (0–74)	28.1 $\pm$ 42.5 (11–369)

There was no difference in neutrophil or platelet engraftment after changing the autologous stem cell graft thawing procedure from post-thaw washing in the laboratory to bedside thawing. Bedside thawing of stem cells is a safe procedure that results in acceptable cellular engraftment.

**Disclosure of conflict of interest:** None.

## Stem cell mobilization, collection and engineering

P026

**10% DMSO in cryoprotective mixture may be reduced to 5% without negative impact on hematopoietic engraftment. Final results of prospective, randomized trial**

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The procedure of autologous hematopoietic stem cell (HSC) transplantation requires cryopreservation of HSCs. Addition of DMSO (dimethyl sulfoxide) is necessary to secure the viability of such cells, but this cryoprotectant causes adverse reaction during infusion into patient. The concentrations of DMSO in cryopreservation mixture vary strongly between different transplant centers. Usually, the HSCs are stored in mixtures containing 10% DMSO, however, many centers successfully use lower concentrations. The main aim of the study was to evaluate the clinical impact of different DMSO concentrations in cryopreservation mixture (5%, 7.5%, 10%) on reconstitution of hematopoiesis after autologous HSC transplantation. The

project was approved by the local Bioethics Committee. Written informed consent obtained from all of patients. The study is registered to ClinicalTrials.gov (identifier: NCT02452099). Between January 2014 and July 2016, 150 consecutive patients with hematological malignancies or solid tumors, referred for autologous HSC transplantation, were recruited in the study. The patients were randomly assigned to one of three study arms (50 patients each). HSCs obtained by leukapheresis were cryopreserved in three concentrations of DMSO: 5%, 7.5%, 10%, respectively. Study groups did not differ significantly with regard to the diagnosis (mostly MM, NHL or HL), age or conditioning regimen (chemo- or radiotherapy-based). All patients received granulocyte-colony stimulating factor (G-CSF, filgrastim) starting from day +4 after transplantation to support neutrophil recovery. In case of 7 patients, the transplantation was cancelled due to progression or other medical reasons. Four patients died shortly after transplantation, due to refractory infections. Data for 139 patients were subjected to statistical analysis. The viability of nucleated cells on the day of transplantation was similar in all groups (median 96%, range: 85–99% for 10% DMSO group; 97%, range: 85–99% for 7.5% DMSO; 97%, range: 90–99% for 5% DMSO;  $P=0.52$ ). The dose of transplanted CD34+ cells was comparable in all group: (median  $4.70 \times 10^6/\text{kg}$  of recipient body weight for 10% DMSO,  $4.35 \times 10^6/\text{kg}$  for 7.5% DMSO and  $3.97 \times 10^6$  for 5% DMSO,  $P=0.44$ ). The median time to leukocyte recovery, defined as the first day with WBC count exceeding  $1.0 \times 10^9/\text{L}$  was 10 days in all groups (ranges: 9–12 for 10% DMSO; 7–11 for 7.5% DMSO; and 9–12 for 5% DMSO;  $P=0.36$ ). Similar results were obtained in case of neutrophil recovery—the median day, when the ANC exceeded  $0.5 \times 10^9/\text{L}$ , was 10 in all arms (ranges: 9–12; 8–11 and 9–12, respectively;  $P=0.20$ ). The day when the platelets level were greater than or equal to  $20 \times 10^9/\text{L}$  (sustained without transfusion within 7 days) was similar in all groups: medians were 15 days in 10%, 7.5% and 5% DMSO (ranges: 8–20; 0–19; 0–24;  $P=0.61$ ). No serious adverse effects were observed during HSCs infusion and during 24 h after transplantation. Reduction of DMSO concentration from in cryoprotective mixture 10% to 7.5% and 5% has no negative impact on cell viability during cryopreservation and engraftment after auto-HSC transplantation.

**Disclosure of conflict of interest:** None.

P027

**A real-world cost-effectiveness analysis demonstrates that introducing Plerixafor to improve mobilization in multiple myeloma patients who behave as poor mobilizers is cost-effective considering the whole mobilization and transplant procedure**

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Plerixafor, a CXCR4-antagonist, is efficient to improve CD34+ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern. Published medico-economics studies were mostly conducted in the US, and few including detailed and comprehensive micro-costing of the collection and transplantation process; conclusions may thus not apply to European countries where cost structures are different. To compare costs and effectiveness of plerixafor-free and plerixafor-replete management strategies for multiple myeloma patients who behaved as poor-mobilizers after adequate

administration of a standard rhG-CSF mobilization regimen. Sixty patients diagnosed with multiple myeloma were consecutively identified during years 2009–2011, immediately before and after EMA granted marketing authorization for plerixafor. Poor-mobilizers were defined as having circulating CD34+ cell counts below 20/μL. Plerixafor was introduced or not as a result of the attending physician's decision, reflecting progressive changes in medical practices over this transitional period. The historical and study groups were matched over four criteria: disease stage at diagnosis, age, gender and number of chemotherapy treatments received before mobilization. Two cost-effectiveness analyses (CEA) were conducted; the primary CEA looked at the criterion 'collecting at least  $2 \times 10^6$  CD34+ cells'; a secondary CEA looked at the criterion 'successful autologous transplant administered'. Detailed micro-costing evaluations (2015 figures) did not or did include transplantation costs for the first and second CEA, respectively. The two groups were similar in terms of age, sex distribution, disease characteristics or previous treatments. 27/30 and 26/30 patients proceeded to high-dose melphalan and autologous transplantation in the study and historical groups, respectively. There was a trend to a higher number of collected CD34+ cells in the control group; however, the proportion of patients who met the minimal target number of  $2 \times 10^6$  collected CD34+ cells/kg was identical (28/30). Length of hospitalization, times to neutrophil and platelet recoveries, numbers of PRBC and platelet transfusions were identical in the two groups. Mobilization and collection costs per patients were more important in the plerixafor group than in the historical group (8.757 vs 5.460 €,  $P < 0.0001$ ), and proportionally higher in patients who received plerixafor as part of a remobilization treatment rather than pre-emptively (10.401 vs 8.162€, respectively). The main CEA concluded to a 3.237€ increase in costs for the same number of patients achieving a minimal target number of  $2 \times 10^6$  collected CD34+ cells/kg. The second CEA found a decrease in the cost of transplant, with 12.724€ in the study group vs 13.634€ in the historical group (NS). In total, the 2.035€ increase for the complete procedure cost (22.866€ per successfully autografted patient in the study group vs 20.831€ in the historical group) was not statistically different. Cost-effectiveness arguments should not be used against the administration of plerixafor in multiple myeloma patients in the European context. Future prospective researches looking at patients reported outcome criteria and labour organization in apheresis facilities are needed.

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## P028

### Administration of plerixafor for peripheral blood CD34+ stem cell content of $< 30 \times 10^6/L$ for autologous stem cell mobilization leads to decreased apheresis days and increased total yield

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Autologous stem cell transplantation (ASCT) is an effective treatment for lymphoma and plasma cell neoplasm (PCN) (multiple myeloma and amyloidosis). Granulocyte-colony stimulating factor (G-CSF) is the most commonly used upfront mobilizing agent with plerixafor-based higher cost approaches reserved for poor/unsuccessful mobilizers. Several mobilization algorithms utilizing G-CSF and plerixafor have been published however the most efficient and cost effective strategy is yet to be determined. Most transplant centers administer plerixafor for peripheral blood (PB) CD34+ stem cell content of  $< 10 \times 10^6/L$  on day (D) 4 of G-CSF mobilization. At the University of Colorado (UCH) we changed our programmatic approach in 2015 and administered plerixafor for PB

CD34+ count of  $< 30 \times 10^6/L$  on D4 of G-CSF mobilization. In this study we evaluate the impact of this novel mobilization algorithm on apheresis days and total stem cell yield. Patients (pts) with lymphoma and PCN who underwent ASCT at UCH until 3/2015 received plerixafor if PB CD34+ cells on D4 of G-CSF mobilization was  $< 10 \times 10^6/L$ . Based on our institutional review of poor/unsuccessful mobilizers and using logistic regression analysis this algorithm was revised in 4/2015. In the new algorithm all pts received plerixafor if PB CD34+ cells on D4 of G-CSF mobilization was  $< 30 \times 10^6/L$ . Demographics were compared between pts with lymphoma and PCN before (Group 1: 9/2013–3/2015) and after (Group 2: 4/2015–4/2016) the new algorithm was implemented. The primary goal of this analysis was to assess the total days of apheresis and total stem cell yield between the two groups. We also sought to analyze days to WBC engraftment and platelet engraftment. A total of 131 pts were included in this analysis. Group 1 consisted of 77 pts (26 pts had lymphoma and 51 pts had PCN). Group 2 consisted of 54 pts (20 pts had lymphoma and 34 pts had PCN). We found that there was a significant increase in total yield ( $P=0.0017$ ) in Group 2 as compared to Group 1. On further disease subtype assessment we noted that pts with PCN in Group 2 had a significant increase in total yield ( $P=0.0014$ ). In lymphoma pts on univariate analysis Group 2 showed a significant decrease in apheresis days (0.468 days,  $P=0.044$ , 95% CI: (-0.91, -0.026)). On multivariate analysis there was still a marginally significant decrease in Group 2 (0.47 days,  $P=0.052$ , 95% CI: (-0.916, -0.024)) compared to Group 1. In PCN pts on univariate analysis Group 2 showed a significant decrease in apheresis days (0.387 days,  $P=0.025$ , 95% CI: (-0.718, -0.056)). On multivariate analysis Group 2 continued to show a significant decrease in apheresis days (0.426 days,  $P=0.02$ , 95% CI: (-0.772, -0.081)) compared to Group 1. We found no significant difference between the two groups in days to neutrophil engraftment and platelet engraftment. Our analysis showed that a mobilization algorithm of administering plerixafor for a PB CD34+ stem cell count of  $< 30 \times 10^6/L$  on D4 of G-CSF mobilization led to a decrease of roughly 0.46 days in the lymphoma cohort and a significant decrease of 0.43 days in the plasma cell neoplasm cohort. We also noted a significantly increased total yield of stem cell collection in Group 2. Overall our programmatic approach led to decreased chair-time for apheresis and better resource utilization. Pharmacoeconomic impact of this approach will be updated at the meeting.

**Disclosure of conflict of interest:** MK: Speakers Bureau, Seattle Genetics; remaining authors declare no conflict of interest.

## P029

### Previously published

## P030

### Previously published

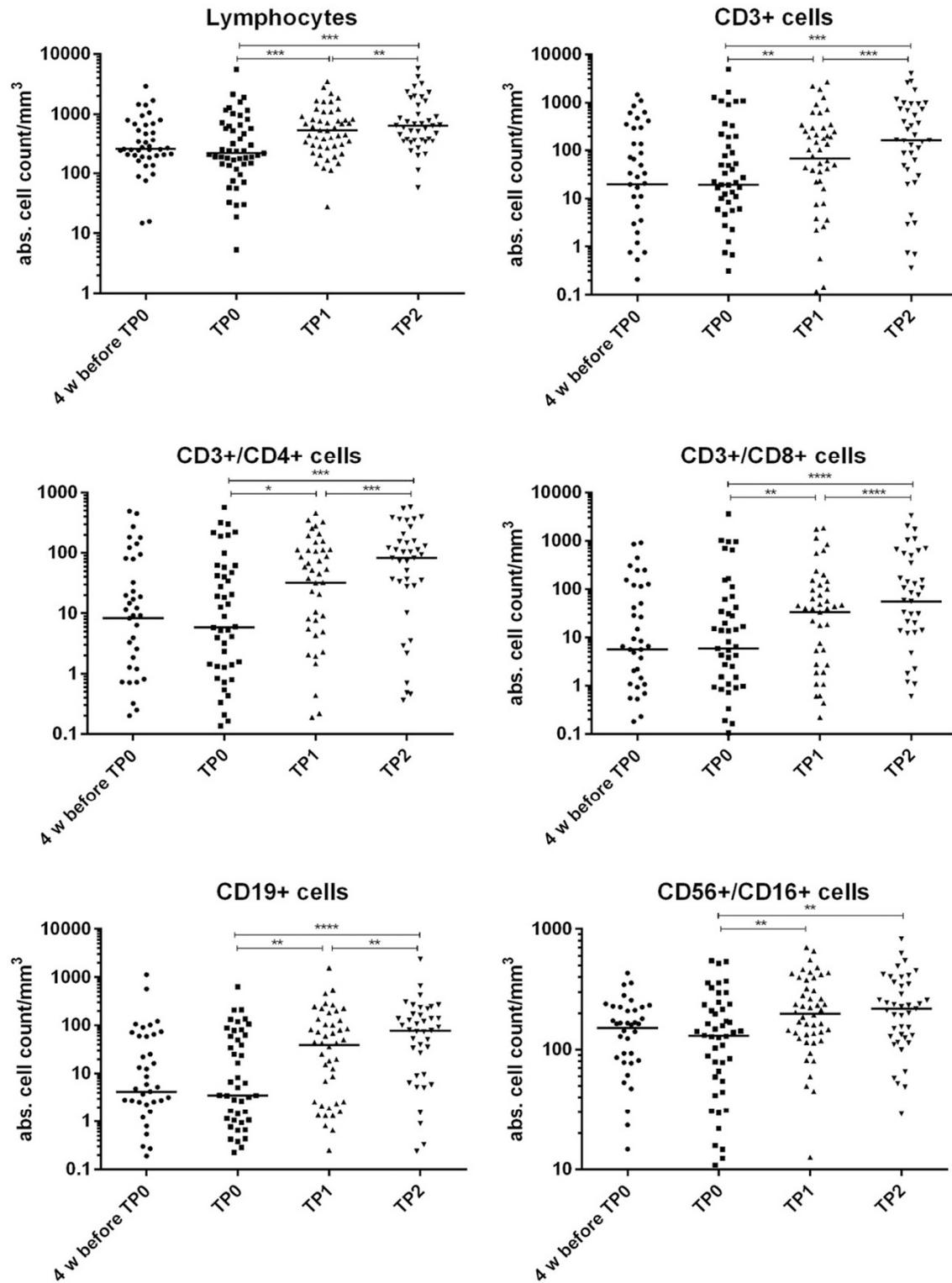
## P031

### CD34+ selected stem cell boosts can improve poor graft function after allogeneic stem cell transplantation in pediatric patients

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Poor graft function (PGF) is a severe complication after hematopoietic stem cell transplantation (HSCT).



Lymphocyte counts 4 weeks prior to, at day of infusion (TP0) and 4 weeks (TP1) / 8 weeks (TP2) after stem cell boosts.

Administration of stem cell boosts (SCBs) from the original donor offers a therapeutic option. We report on 50 pediatric patients with PGF who received a total of 61 boosts with CD34 + selected peripheral blood stem cells (PBSC) after transplantation from matched unrelated ( $n=25$ ) or mismatched related ( $n=25$ ) donors. Median time between HSCT and infusion of the 61 SCBs was 94 days (13–519). Boosts contained a median number of  $3.15 \times 10^6$  CD34+ progenitor cells/kg body weight (range:  $0.71-27.9 \times 10^6$ ) with a median number of 2417/kg (range: 100–23 630) residual CD3+ T cells. Within 8 weeks after application, a significant increase in median neutrophil counts (600 vs  $1516/\text{mm}^3$ ,  $P < 0.05$ ) and a decrease in erythrocytes and thrombocytes transfusion requirement (median frequencies 1 and 7 vs 0,  $P < 0.0001$  and  $< 0.001$ ), were observed, and 78.8% of the patients resolved one or two of their initial cytopenias whereas 36.5% had a complete hematological response. Additionally median lymphocyte counts for CD3+, CD3+CD4+, CD19+ and CD56+ increased 8.3 fold, 14.2 fold, 22.3 fold and 1.6 fold, respectively. The rate of *de novo* acute GvHD grade I–III was only 6% and resolved completely after treatment. No GvHD IV or chronic GvHD occurred. Patients who showed a response to SCB displayed a trend toward better overall survival (OS) ( $P=0.07$ ). Administration of CD34+ selected SCBs from alternative donors is a safe and effective procedure. We hypothesize that the CD34+ progenitor boosts may have an enhancing effect on maturation of committed lymphoid precursors already present in the host or generate another wave of thymic seeding with accelerated T-cell differentiation process in the absence of any immune suppression. Further studies are warranted to better define the impact on immune reconstitution and survival. **Disclosure of conflict of interest:** None.

### P032

#### Combination of plerixafor and G-CSF for mobilization: Impact on engraftment in autologous stem cell transplantation for multiple myeloma

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[P032]

Median days to reach:	P/G-CSF (range)	G-CSF (range)	p Value
Neutrophils $\geq 500/\mu\text{L}$	12 (8-14)	12 (11-24)	Not significant (NS)
Neutrophils $\geq 1.000/\mu\text{L}$	12 (9-15)	12 (11-24)	NS
Platelets $\geq 20.000/\mu\text{L}$	14 (10-28)	13 (9-41)	NS
Platelets $\geq 50.000/\mu\text{L}$	17 (14-60)	16.5 (12-60)	NS
Median number of transfused units:			
Red blood concentrates	2 (0-6)	2 (0-6)	NS
Pooled platelets	4 (1-14)	3 (1-17)	NS
Median days of hospital stay:	19 (3-29)	18 (17-31)	NS

Plerixafor plus granulocyte-colony stimulating factor (G-CSF) has been shown to mobilize more CD34+ cells than G-CSF alone for autologous hematopoietic stem cell transplantation (HSCT). However, there are few studies that analyze the impact of this strategy in engraftment. The aim of our study is to compare mobilization and engraftment between patients who received a combination of Plerixafor plus G-CSF and patients (pts) who mobilized with G-CSF alone. A retrospective case-control analysis was performed in 24 pts with myeloma who mobilized with Plerixafor plus G-CSF (group P/G-CSF) and was compared with 24 matched for sex and age controls who mobilized with G-CSF alone (group G-CSF). All pts underwent HSCT between 2009 and 2015. Mobilization with G-CSF at dose of  $10 \mu\text{g}/\text{kg}/\text{day}$  was used in all pts. The aphaeresis was scheduled on day +5. Plerixafor ( $0.24 \text{ mg}/\text{kg}$ ) was added if the number of CD34+ cells on day +4 was  $< 10/\mu\text{L}$  for  $2 \times 10^6$  CD34+/kg requested (or  $< 20/\mu\text{L}$  for  $4 \times 10^6$  CD34+/kg), or if the number of CD34+ cells collected in the first aphaeresis was  $< 50\%$  of CD34+ requested. Conditioning and supportive care were similar in both groups. In P/G-CSF group, 13 were male and 11 female. Median age was 60.92 years (range: 49–71). In group G-CSF, 15 were men and 9 female. Median age was 60.67 years (range: 50–73 years). There were no differences between both groups. Disease status at time of mobilization was different between groups ( $P=0.023$ ). In P/G-CSF group: 11 (45.83%) pts were in complete remission (CR), 4 (16.66%) very good partial responses (VGPR), 7 (29.16%) partial response (PR) and 2 (0.08%) had no response to treatment. In G-CSF group: 7 (29.16%) pts had reached CR, 13 (51.16%) VGPR and the remainder in PR. Sixteen (66.67%) pts in P/G-CSF group had received  $\geq 2$  lines of treatment vs 9 (37.5%) pts in G-CSF group ( $P=0.046$ ). No difference was seen on mean day-dose of G-CSF ( $14 \mu\text{g}/\text{kg}/24 \text{ h}$  in P/G-CSF group vs  $12 \mu\text{g}/\text{kg}/24 \text{ h}$ ) ( $P=0.067$ ). There was no difference on CD34+/kg requested (19/24 pts in P/G-CSF were requested  $2 \times 10^6/\text{kg}$  vs 18/24 in G-CSF group) ( $P=0.73$ ). P/G-CSF group needed more aphaeresis sessions, 17 (70.83%) pts required  $\geq 2$  sessions against 4 (16.67%) pts in group G-CSF ( $P < 0.001$ ). We obtained enough CD34+ cells to carry out HSCT in all patients, although mean number of CD34 + cells obtained in p/G-CSF group was lower than in G-CSF group ( $2.92 \times 10^6/\text{kg}$  vs  $4.98 \times 10^6/\text{kg}$ , respectively) ( $P < 0.001$ ). Also, mean number of CD34+ infused in P/G-CSF group was lower ( $2.92 \times 10^6/\text{kg}$  vs  $3.55 \times 10^6/\text{kg}$ ) ( $P < 0.001$ ). However, engraftment results were similar in both groups, as represented in Table 1. Patients who required mobilization with Plerixafor plus G-CSF got an engraftment as good as patients who do not require the combination despite of worse baseline parameters. Given that the number of CD34+ infused in the P/G-CSF group has been lower than G-CSF group, these results might suggest that the different composition of graft cell with Plerixafor plus G-CSF mobilization, described in some studies, could impact on engraftment outcomes.

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**Disclosure of conflict of interest:** None.

Median days to reach:	P/G-CSF (range)	G-CSF (range)	p Value
Neutrophils $\geq 500/\mu\text{L}$	12 (8-14)	12 (11-24)	Not significant (NS)
Neutrophils $\geq 1.000/\mu\text{L}$	12 (9-15)	12 (11-24)	NS
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Median days of hospital stay:	19 (3-29)	18 (17-31)	NS

**P033**

**Effects of plerixafor in the lymphocyte count in aphaeresis product**

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Day 15 absolute lymphocyte count (ALC-15) after autologous peripheral blood hematopoietic stem cell transplantation (APHSCT) has been reported to be a significant predictor for survival in acute myeloid leukemia, Hodgkin's and non-Hodgkin's lymphoma (NHL) multiple myeloma (MM), primary systemic amyloidosis and breast cancer<sup>1</sup>. On the other hand, use of Plerixafor has been related with increase of CD3+ cells in aphaeresis product. Corroborate if there is any correlation between the use of Plerixafor and lymphocyte mobilization. Assess the relation of the lymphocyte count in the product and the ALC-15 after AHSCT. We compared patients with MM and NHL mobilized with Plerixafor + granulocyte-colony stimulating factor (G-CSF) from January 2015 to May 2016 with a control group of patients mobilized with G-CSF alone. G-CSF doses were 5 µg/kg/12 h subcutaneous. It was added Plerixafor (0.24 mg/kg) if the count of CD34+ cells on day +4 of mobilization was < 10/µL, according with our hospital protocol. We determinate the counting of CD34+ and CD3+ cells by flow cytometry. Thirty-nine patients were included in our study. Eighteen were diagnosed of NHL and twenty one of MM. Plerixafor + G-CSF were used in 13 patients (eight were NHL and five were MM) and G-CSF alone was used in 26 (10 were LNH and 16 were MM). There were no differences on mean age, sex or disease status between groups. We found no association between lymphocyte count in the product and the ALC-15 (P>0.5). Mean CD3+ in product was 266.28 for Plerixafor + G-CSF group and 248.74 for G-CSF alone group. Mean ALC-15 for Plerixafor + G-CSF was 692 and for G-CSF was 819.6, respectively. Results for each disease can be visualized in Table 1.

[P033]

Table 1. Results of CD3+ cell in product.

Diagnosis	Mobilization	n	CD3+ x 10 <sup>6</sup> /kg (mean ± DS)	P
NHL	G-CSF	8	171.18 (± 145.47)	0.017
	Plerixafor+ G-CSF	10	205.89 (± 145.472)	
MM	G-CSF	4	440.16 (± 197.52)	0.063
	Plerixafor+ G-CSF	16	278.89 (± 94.45)	

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**Disclosure of conflict of interest:** None.

**P034**

**Efficacy and factors influencing peripheral blood stem cell mobilization and collection for the autologous transplantation in patients with lymphoma**

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High-dose chemotherapy following autologous hematopoietic stem-cell transplantation (autoHSCT) is an effective method of

treatment both recurrent and primary refractory lymphoma patients. However, some patients have mobilization failure ('poor mobilizers') with inadequate collection of peripheral blood stem cell (PBSC). **Aim:** To evaluate the efficacy and factors influencing PBSC mobilization and collection for the autoHSCT in patients with lymphomas. Thirty patients were included in this study: 17—with Hodgkin lymphoma, 7—with non-Hodgkin lymphoma, 6—with multiple myeloma; 17 women and 13 men of them. The median age of patients was 36 years (24–64 years). The mobilization of PBSC with only colony-stimulating factors (CSF) was carried out for 17 patients, chemotherapy (cyclophosphamide, etoposide) in combination with CSF—for 13 patients. Only one patient had plerixafor mobilization. The concentration of CD34+ in peripheral blood (PB) was studied on the day of the intended cytapheresis. Cytapheresis was commenced when CD34+ concentration had been greater than 0.01×10<sup>6</sup> cells/mL. Twenty-four patients (80%) from 30 had collection of PBSC. The collection was not performed in six patients (20%) because the concentration of CD34+ in PB on the day of the intended cytapheresis was lower than 0.01×10<sup>6</sup> cells/mL. There was no possibility to use plerixafor in these cases for economic reasons. The median concentration of CD34+ in PB on the first day of the intended cytapheresis in the group of patients that had cytapheresis was 0.013×10<sup>6</sup> cells/mL whereas in the group of failed—0.005×10<sup>6</sup> cells/mL (P<0.05). Fifty-nine tests of CD34+ in PB were done. Distribution and test results by days from the first day of the intended cytapheresis are presented in Table 1. The total number of the cytapheresis was 36. The majority of patients had 1 procedure of PBSC collection (n=22), 13 patients had 2 procedures and only 1 had 3. The last patient had had two previous failed cytapheresis procedures and the adding of plerixafor helped him to collect necessary number of cells. The median of CD34+ cells on patient's kilo was 2.85×10<sup>6</sup>cells/kg. Sex, age, mobilizing regimen, previous radiation therapy, the count of lines of chemotherapy before autoHSCT were not significantly associated with poor PBSC mobilization and collection. Only tumor response before autoHSCT (complete/partial response or stabilization) was significantly associated with CD34+ cell count in the product of cytapheresis. Patients with complete or partial response had significantly better CD34+ count.

[P034]

Table 1. The concentration of CD 34+ (x10<sup>6</sup> cells/ml) in peripheral blood

	N	The average value	The median value	Minimum	Maximum
CD 34+ in PB on the 1st day of the cytapheresis	22	1.78	0.94	0.35	9.08
CD 34+ in PB on the 2nd day of the cytapheresis	13	1.18	0.64	0.25	4.06
CD 34+ in PB on the 3rd day of the cytapheresis	1	0.32	0.32	0.32	0.32

**Disclosure of conflict of interest:** None.

**P035**

**Factors associated with failure in mobilization of peripheral blood hematopoietic progenitor cells in autologous transplantation**

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High dose therapy followed by autologous stem cell transplantation (ASCT) obtained from peripheral blood is currently the standard model for treatment consolidation in various hematologic malignancies. A global incidence of 5–40% of failure to mobilization is reported, and some factors associated with poor mobilizers in Hodgkin's Lymphoma (HL), Non-Hodgkin's Lymphoma (NHL) and Multiple Myeloma (MM)

**Table 1. Multivariate analysis for predictive factors of poor mobilization.**

	Multiple Myeloma			Non-Hodgkin's Lymphoma			
	OR	IC 95%	p	OR	IC 95%	p	
Bone marrow fibrosis	12.4	1.77-86.5	0.013	-	-		
Hemoglobin at diagnosis <10 g/dl	8.33	1.4-46.7	0.019	-	-		
Platelets at diagnosis <150000/ $\mu$ l	6.0	1.3-25.85	0.018	4.2	1.3-13.47	0.022	

such as induction therapy, radiotherapy, bone marrow cellularity and cytopenia prior to cell collection are reported. A total of 152 patients were evaluated from January 2010 to December 2015. G-CSF (Filgrastim), CFM 1.5 g/m<sup>2</sup> + G-CSF, and Pegfilgrastim were used as the mobilization agent. Cell collection was performed with Spectra Optia equipment. Poor mobilization was defined as the failure to collect a minimum of 2 × 10<sup>6</sup> CD34+ cells/kg body weight during the first three sessions of leukapheresis. We analyzed the results of 152 patients, with a diagnosis of MM (40%), NHL (43%), and HL (17%). Predominance of male gender (57%). The median age was 46.5 years (17–72), of which 26% presented comorbidity at the time of diagnosis. Poor mobilizers were 28.3%, of which corresponds to NHL 58%, MM 33% and 9% with HL. In the bivariate analysis in MM predictive factors of poor mobilization were the previous comorbidity (P = 0.034) Hemoglobin (Hb) at diagnosis (P = < 0.001) pre-harvest platelet count (P = < 0.001), pre-count CD34+ cells/ $\mu$ L (P = < 0.001). In LH, there were no risk factors associated with poor mobilization. In the case of NHL, the platelet count at diagnosis (P = 0.022) and pre-harvest (P = < 0.008), the pre-count CD34 +/ $\mu$ L (P = < 0.001) was significant. The multivariate analysis for predictive factors of failure to mobilization is shown in Table 1. The most important risk factors for poor mobilization are the underlying pathology, NHL is the most frequent followed by MM, the risk factors identified for poor mobilization in MM were the presence of Bone marrow fibrosis, Hemoglobin at diagnosis

**Disclosure of conflict of interest:** None.

**P036**

**G-CSF mobilized peripheral blood stem cell collection for allogeneic transplantation in healthy donors: Analysis of factors affecting yield**

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When the yield in peripheral blood stem cells (PBSC) collection is unsatisfactory, the effects for the recipient can be serious. The donor's age, gender, body surface area (BSA), processed blood volume and the method of G-CSF dose calculation may affect the CD34+ yield. As G-CSF has a low distribution volume in the peripheral blood (PB), it might be appropriate to

calculate the doses by using the BSA instead of per kg body weight. 175 consecutive allogeneic PBSC donations performed in 170 healthy donors at the Karolinska University Hospital in Stockholm were included. A complete medical history, physical examination, electrocardiogram, chest x-ray and laboratory testing were done before PBSC donation. Relevant data for analysis were collected from the institutional quality database for a retrospective review. The total blood volume was calculated using the formula by Nadler *et al.* The BSA was calculated using the formula by Du Bois and Du Bois. The concentration of CD34+ cells in the PB and the processed volume of blood were significantly correlated to CD34+ cells yield (P < 0.00005 and P < 0.001, respectively, see Table 1). The G-CSF dose per m<sup>2</sup> was significantly correlated to the concentration of CD34+ cells in the PB (P = 0.0003) and in the product (P = 0.01, see Table 1). Smaller BSA (P < 0.001) and less processed volume (P < 0.001) were found among female donors, who were given lesser G-CSF dose per m<sup>2</sup> (P < 0.001) and showed lower yield compared to men (P < 0.05). However, multivariate analysis of the yield showed that only the concentration of CD34+ cells in the PB and the processed volume remained independent significant (see Table 1).

[P036]

TABLE Factor	Yield with respect to the number of CD34+ cells/kg donor		Yield with respect to the number of CD34+ cells in the product	
	Univariate analysis, p	Multivariate analysis, p	Univariate analysis, p	Multivariate analysis, p
Male sex	<0.05	NS	0.001	NS
Body weight	0.157	NS	<0.00005	NS
Body surface area (BSA)	0.162	NS	<0.00005	NS
G-CSF dose/kg	0.990	ND	0.996	ND
G-CSF dose/m <sup>2</sup>	0.397	ND	0.011	NS
Concentration CD34+ cells x 10 <sup>6</sup> /L in PB	<0.00005	<0.00005	<0.00005	<0.00005

In this study, we found the concentration of CD34+ cells in the PB and the processed volume of blood to be independent predictors of yield. We recommend to get a high concentration of CD34+ cells in the PB, and to process adjusted volumes of blood when needed. An evaluation if the calculation of G-CSF dose per m<sup>2</sup> is more appropriate than per kg body weight should be done in future studies.

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**Disclosure of conflict of interest:** None.

Table 1

		Diagnosis								Total		
		MM		NHL		HL		AML			others	
LAs Patients	Number n (%)	80	28%	133	41%	22	6%	32	16%	23	8%	290
	Age, median (range)	60	(41-76)	56	(19-65)	42	(18-65)	51	(18-70)	45	(41-64)	
ASCT	Patients	191		190		45		19		13		458 patients for a total of 510 ASCT

### P037

#### Graft purity and composition significantly impact the engraftment of autologous stem cell transplants

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Autologous stem cell transplantation (ASCT) has been widely used in the treatment of hematological malignancies over the last two decades. Despite its broad use, some characteristics that might influence engraftment have not been exhaustively investigated, particularly graft purity with respect to contamination by platelets (PLTs) and White Blood Cells (WBC). Here we report collection characteristics and engraftment kinetics of a single Center consecutive series of 510 ASCT. We retrospectively collected clinical records of 481 patients who underwent leucapheresis procedures (LA; followed or not by ASCT) and data on 510 ASCT at our Institution over 16 years (2000–2016) (Table 1). The impact on engraftment kinetics of conditioning chemotherapies, amount of infused CD34+ cells and WBC/PLTs graft contamination were analyzed. Absolute neutrophil count (ANC) engraftment was defined as the duration of neutropenia (from day 0 to the first of 3 consecutive days of ANC > 500/μL post ASCT). Regarding CD34+ cell collection, no impact of mobilizing regimens and WBC count during LA was observed. On the other hand, we observed a difference in the number of total CD34+ cells collected among different diagnoses: the median overall collection was 7.2 (0.65–64.06) × 10<sup>6</sup>/kg CD34+ cells for NHL patients, 5.66 (0.71–23.31) × 10<sup>6</sup>/kg for MM patients, 6.15 (0.51–23.24) × 10<sup>6</sup>/kg for HL patients and 3.56 (0.64–20.3) × 10<sup>6</sup>/kg for AML patients) (*P* = 0.001). Considering CD34+ cells/kg harvested on the first day of LA, 59.2% of NHL and HL, 57.5% of MM patients and 34% of AML patients harvested ≥ 5 × 10<sup>6</sup>/kg CD34+ cells. Of note, among AML patients, 40.6% collected < 2.5 × 10<sup>6</sup>/kg. The differences were statistically significant (*P* = 0.003). Moreover, an inverse correlation between collected CD34+ cells and age was shown (*P* = 0.001). ANC recovery after ASCT was not influenced by conditioning regimen whereas diagnosis impacted on the duration of neutropenia (AML patients displayed a longer aplasia, *P* < 0.01). We observed that the median days with ANC < 500/μL were 10, 11 and 12 in patients who received > 5.3 × 10<sup>6</sup>/kg, 3.5–5.3 × 10<sup>6</sup>/kg and < 3.5 × 10<sup>6</sup>/kg CD34+ cells, respectively (*P* < 0.0001). Furthermore, the same finding was observed considering the duration of thrombocytopenia (median number of days with PLTs < 50 000/μL: 15, 18 and

20 in patients who received > 5.3 × 10<sup>6</sup>/kg, 3.5–5.3 × 10<sup>6</sup>/kg and < 3.5 × 10<sup>6</sup> CD34+ cells, *P* < 0.0001). Looking at the apheresis product, we analyzed the impact of harvest contaminating WBC and PLTs on engraftment kinetics. Notably, when the ASCT collection contained > 100 × 10<sup>3</sup>/μL WBC, ANC engraftment (days with ANC < 500/μL) lasted longer (median days 11) compared to patients who received a graft with lower WBC count (*P* < 0.0001). A faster ANC engraftment was also observed in patients receiving harvests with PLTs levels > 600 × 10<sup>3</sup>/μL compared to those who infused a collection bag with PLTs < 600 × 10<sup>3</sup>/μL (*P* = 0.005). Herein, we confirmed that the disease and the amount of infused CD34+ cells significantly influence time of ANC and PLTs engraftment; furthermore, we observed for the first time that quality and purity of the graft have a substantial impact on engraftment kinetics.

**Disclosure of conflict of interest:** None.

### P038

#### Hematopoietic stem cell mobilization: Predicting the unpredictable

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A combination of chemotherapy with growth factor is a commonly used strategy for Hematopoietic stem cell (HSC) mobilization. The collection of timely and adequate numbers of HSCs is a prerequisite for proceeding to transplantation. A variety of mobilization strategies are currently used. The knowledge of efficacy, safety and predictability of different HSC mobilization strategies might help blood and marrow transplantation (BMT) programs to effectively schedule patients for mobilization. Given the many variables associated with the mobilization of HSC, collecting an adequate stem cell dose in a timely and effective manner is an art and science. Factors that might affect the process includes type of disease and mobilization protocol, financial clearance, availability of chemotherapy beds, scheduling various diagnostic procedures and transplant urgency. To evaluate the effectiveness and related coordination efforts of 'just-in-time' strategy of HSC mobilization and collection, we performed a retrospective study comparing all patients in whom peripheral HSC mobilization was attempted at KHCC from January 2005 through November 2016. Data collected included the disease type, mobilization protocol, days to and number of collections, CD34+ cell dose, calendar of the mobilization and collection. The records of a total of 1042 mobilizations were reviewed. 364 were of healthy allogeneic donors, and the remaining 678 were of patients undergoing autologous transplantation. Table 1 depicts the overall summary of number of days and collection procedures per each protocol. Detailed mobilization kinetics per disease type and mobilization protocol were also captured and evaluated.

[P038]

Mobilization Protocol	# Patients	% of Total Mobilizations	# Collections	Mobilization Days ± SD	#PBSC /Patient
G-CSF (Allogeneic donor)	364	34.9	425	4.7 ± 0.7	1.2
G-CSF (autologous)	48	4.6	100	5.3 ± 0.9	2.1
CY+ G-CSF	306	29.4	468	10 ± 2.1	1.5
DHAP + G-CSF	233	22.4	301	10.2 ± 2	1.3
ICE + G-CSF	71	6.8	110	12 ± 2.7	1.5
Plerixafor + G-CSF	20	1.9	38	5.6 ± 0.8	1.9
<b>Total</b>	<b>1042</b>	<b>100%</b>			

Detailed analysis of mobilization kinetics comparing different mobilization strategies aids in prediction of number of days of mobilization and anticipated number of collections. This helps in proactively scheduling patients based on collection predictability. A seamless communication through a shared calendar between key parties, primarily BMT physicians and nurse coordinators, BMT and flow cytometry laboratories and chemotherapy unit can be achieved.

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**Disclosure of conflict of interest:** None.

**P039**

**Impact of lenalidomide induction in the mobilization of CD34+ cells, blood graft cellular composition and post-transplant recovery in myeloma patients: a prospective multicenter study**

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Autologous stem cell transplantation is still a standard of care in the treatment of multiple myeloma. Lenalidomide-based regimens are commonly used in both transplant-ineligible as well as -eligible patients. Prolonged lenalidomide-exposure is known to affect mobilization of CD34+ cells, although the basic mechanisms are poorly understood. Limited prospectively collected data is available on the effect of lenalidomide in the capacity to mobilize CD34+ cells for transplantation as well as graft cellular composition and post-transplant hematological recovery compared to the lenalidomide-naive patients. This prospective study included 60 newly diagnosed myeloma patients who received mobilization with low-dose cyclophosphamide + G-CSF, were successfully apheresed and transplanted before the end of 2014. Twenty-six patients had received a median of three cycles of lenalidomide-based induction (43 %), whereas 34 patients were lenalidomide-naive and served as the control group. Both baseline characteristics and collection targets were similar between the groups. CD34+ mobilization and apheresis yields were analyzed and compared between the groups. Blood graft cellular composition was analyzed from the thawed cryopreserved samples with a

flow cytometry. Graft function was evaluated by collecting engraftment data as well as by total blood counts at day +15 and at 1, 3, 6 and 12 months after post-transplant. The patients in the lenalidomide group had both lower median peak B-CD34+ counts and about 40% lower CD34+ yields of the first apheresis but without statistical significance (Table 1). The median number apheresis was significantly higher in the lenalidomide arm (2.0 vs 1.5, P=0.039). The number of CD34+CD133+CD38-, CD3+CD4+, CD3+CD8+ cells and NK cells in the cryopreserved grafts were comparable between the arms. Time to neutrophil engraftment was 12 days in the both groups. The median time to platelet engraftment was 12 d in the lenalidomide group and 13 d in the control group. Hematological recovery was comparable between the groups within 12 months post-transplant. Lenalidomide-based induction therapy seems to have an impact in the number of apheresis needed, but not in the total yield of CD34+ cells in the graft. Neither the graft cellular composition nor post-transplant recovery in myeloma patients was affected by the limited duration of lenalidomide used before mobilization and collection of blood grafts.

[P039]

Table1. Mobilization and harvesting results in myeloma patients according to the previous lenalidomide use

Variable	LEN(+) n=26	LEN(-) n=34	p-value
Peak B-CD34+ cell count x10 <sup>6</sup> /L, mean(range)	85(12-291)	122(17-415)	0.477
Peak CD34+ count >100x10 <sup>6</sup> /L, N (%)	5(19)	15(44)	0.333
Peak CD34+ count <20x10 <sup>6</sup> /kg, N (%)	1(3)	3(8)	0.261
B-CD34+ cells x10 <sup>6</sup> /L at the time of first apheresis, mean (range)	73(13-291)	104(13.4-415)	0.391
CD34+ cell yield x10 <sup>6</sup> /kg with first apheresis, mean (range)	4.2(0.9-14.7)	7.0(0.8-17.8)	0.362
Total yield CD34+ cells x10 <sup>6</sup> /kg harvested, mean (range)	7.1(2-14.7)	8.5(2-17.8)	0.854
CD34+ cells yield > 4x10 <sup>6</sup> /kg, N (%)	21(80)	28(82)	0.821
CD34+ cells yield > 6x10 <sup>6</sup> /kg, N (%)	12(46)	21(61)	0.663
The number of apheresis, mean (range)	2.0(1-4)	1.5(1-3)	0.039

**Disclosure of conflict of interest:** None.

**P040**

**Large unstained cell percentage as a predictor of efficacious peripheral stem cell mobilization in autologous stem cell transplantation**

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Large unstained cells(LUC) are large peroxidase-negative cells that are displayed on automatic cell counters and present large lymphocytes, virocytes, blasts, hematopoietic stem cells and another abnormal cells. CD34 positive cell count by flow cytometry is routinely used for the prediction of successful peripheral stem cell collection. In this study we evaluated LUC number and percentage as a predictor of successful peripheral stem cell collection in patients who proceeded to autologous stem cell transplantation. LUC number and percentage has been studied for last 2 years in our institute. We evaluate 89 patients who were performed peripheral stem cell collection

between September 2015 and November 2016. Siemens Hematek 3000 system was used for LUC count. LUC numbers and percentage was measured before leukapheresis. We used Pearson test for the correlation and ROC curve for cut off value. Patients' characteristics were shown in Table-1. There was not a correlation between LUC number and mobilized CD34 positive stem cell number. But LUC percentage was positively correlated with mobilized stem cell number ( $P:0.01$ ). A count of  $5 \times 10^6/\text{kg}$  collected stem cells are optimal for autologous stem cell transplantation. We found 2% LUC percentage as a cut-off value for prediction of collecting optimal number of stem cells with 61% sensitivity and 60% specificity. As expected LUC percentage was negatively correlated with white blood cell count. There was no correlation between mobilized CD34 positive stem cell number and age. Both LUC percentage and mobilized CD34 positive stem cell number did not differ with underlying disease. We found only one study in the literature that evaluated LUC percentage as a tool for the prediction of successful stem cell collection. They found that baseline LUC numbers negatively correlated with stem cell mobilization in healthy donors (1). But we measure LUC on apheresis day and found a positive correlation between LUC percentage and stem cell mobilization. And we found a cut-off value for optimal stem cell mobilization with acceptable sensitivity and specificity. In our study we demonstrate that LUC percentage measurement on apheresis day may be a very simple and cheap tool for the prediction of optimal stem cell mobilization.

**Disclosure of conflict of interest:** None.

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[P040]

Table 1: Patients' characteristics

	n=
Age(median)	56(20-75)
Sex(male/female)	56/33
Underlying disease(Multiple Myeloma/Non-Hodgkin Lymphoma/testis tumor/Hodgkin Lymphoma)	67/14/3/5
Mobilization regimen (G-CSF / cyclophosphamide+ G-CSF / G-CSF+plerixafor/ chemotherapy+G-CSF)	75/6/5/3
CD34+ stem cell count(median)	4.56 (0.51-32) $\times 10^6/\text{kg}$
LUC number(median)	0.55 (0.13-6.68) $\times 10^9/\text{L}$
LUC%(median)	2(0.6-13.4)

**P041**

Previously published

**P042**

Previously published

**P043**

**Performance evaluation of the spectra optia apheresis system mononuclear cell collection for autologous peripheral stem cell transplantation**

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The Spectra Optia (SO) apheresis system performs a wide range of therapeutic procedures, including peripheral blood stem cell (PBSC) collection in mobilized donors and patients (pts). The device was studied to evaluate the cellular

composition of PBSCs harvested in pts with multiple myeloma (MM), non Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL) planned for autologous peripheral stem cell transplantation (APBSCT), and to optimize the collection of PBSCs using the CD34+ precourt and collection efficiency (CE2) of apheresis device which is calculated as follows:  $\text{CE2} = \frac{\text{total CD34+ cells collected} \times 10^6/\text{kg}}{\text{CD34+ precourt}/\mu\text{L} \times \text{blood processed (liters)}}$ . The blood volume processed is calculated as follows:  $\text{Desired CD34+} \times 10^6/\text{kg} \times \text{recipient weight (kg)}$ ;  $\text{CE2} \times \text{CD34+ precourt}/\mu\text{L}$ . In our study enrolled pts undergoing PBSC mobilization and planned for APBSCT. We evaluated SO system's mononuclear cell (MNC) collection performance, with respect to CD34+ cells and MNC collection efficiency, platelet reduction pre to post apheresis, and product purity in view of using prediction algorithms to optimize the procedure and predict the CD34+ yield, blood volume processed and platelets loss. We also evaluated neutrophil and platelet recovery in pts who underwent APBSCT. **Results:** Between 30/3/2015 and 30/11/2016, 45 pts underwent PBSC harvesting by SO device. Median age was 46 years (20–71). There were 19 females and 26 males. Diagnosis was MM in 21 pts, HL in 17 pts and NHL in 7 pts. The number of aphereses procedures was 59. Mobilization consisted in G-CSF alone in 36 pts, chemotherapy and G-CSF in 8 pts, and G-CSF + CXCR4 inhibitor in one patient. Median count of CD34 + cells pre-collection was 58/ $\mu\text{L}$  (16.5–372). Median total blood volume processed was 12.4L (6.3–19.9). Median count of CD34 + cells collected was  $4.1 \times 10^6/\text{kg}$  (1–23.6). Median MNC collection efficacy was 48% (7–95). Median CD34+ cell collection efficacy was 45.5% (15–95%). Median platelet reduction pre to post apheresis was 30% (0–50%). Median product hematocrit and granulocytes product was 5% (3–9) and 52% (5–93), respectively. Twenty-six of the 45 pts underwent myeloablative high dose chemotherapy followed by APBSCT which was performed for MM in 18 pts, HL in 6 pts, and NHL in 2 pts. The median count of CD34+ cells infused was  $2.5 \times 10^6/\text{kg}$  (1.15–10.6). All the pts received G-CSF post-APBSCT until neutrophil recovery. The median day for neutrophil recovery was 10 (8–14). Median duration of severe neutropenia ( $\text{ANC} < 0.5 \times 10^9/\text{L}$ ) was 7 days (4–10). The median day for platelet recovery was 10 (7–17). Median duration of severe thrombocytopenia (platelets  $< 20 \times 10^9/\text{L}$ ) was 5.5 days (2–14). **Conclusion:** The study results confirm that the SO apheresis system's MNC collection protocol is safe and effective. The neutrophils and platelets recovery in pts auto-transplanted was not inferior compared to historical controls. In addition, this system help to use prediction algorithms for whole blood processing to achieve a desirable and optimal yield based on CD34+ precourts and CE2 of the apheresis device.

**Disclosure of conflict of interest:** None.

**P044**

**Peripheral blood stem cell apheresis in small children is difficult!**

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In low-weight children with cancer and healthy donor children, peripheral blood progenitor cells (PBPCs) have largely replaced bone marrow as source of autologous and allogeneic stem cells in part because of their relatively easy collection. However, there is a concern regarding medical, psychosocial and technical difficulties in small children. We retrospectively analyzed peripheral blood stem cell apheresis in 38 collections. 30 patients were with cancer (17 patients = Neuroblastoma, 4 patients = Retinoblastoma, 5 patients = Germ cell tumor, 1 patient = Hepatoblastoma, 3 patient = Wilm's tumor) and 8 healthy children donors. The study was conducted between 2012 and 2016. Peripheral stem cell apheresis was performed in the Mahak cancer children's hospital in a nice room for children where the patients stayed with their families. Patients

were not routinely sedated. PBPC were collected by a COBE Spectra cell separator (COBE, Denver, CO, USA). Harvesting was performed after 5 days mobilization. Mean body weight was 11.6 kg (range: 8–15 kg) for a median age of 3 years (range: 10 months–5 years). Mean duration of harvesting was 205 min (range: 164–270 min). Mean volume of stem cell collection was 135 mL (range: 110–240 mL). The mean number of total nucleated cells collected was  $5.4 \times 10^8$ /kg (range:  $3.2$ – $9.9 \times 10^8$ /kg recipients). No side effects occurred. Children didn't require an additional haematopoietic progenitor mobilization or additional apheresis in other day. PBSC collection was without transfusion in healthy donor children. PBSC collection may be difficult in small children owing to the large volume apheresis compared to the child's weight. Various problems, such as metabolic or haemodynamic disorders may be were seen. Peripheral Stem cell harvest can be performed in low-weight children under safe and effective conditions even when systematic priming by blood is avoided. Processing with increase of blood volume may to increase in the yield by recruiting progenitor cells.

**Disclosure of conflict of interest:** None.

#### P045

##### Peripheral blood stem cell collection in low body weight children: A single centre experience

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PBSC became preferred source for autologous transplantation because of easier collection and faster engraftment. However apheresis for low body weight children (< 12.5 kg) is affected by some issues: venous access, extracorporeal volume, metabolic and hemodynamic complications, citrate toxicity, so is crucial to standardize harvesting procedure both maximizing stem cells collection and reducing adverse events. A dual lumen central venous catheter was used to obtain a minimal blood flow of 10–15 mL/min and PBSC collection was performed with Spectra Optia MNC v6.1 apheresis system, starting with CD34+ cell  $\geq 20 \mu\text{L}$  in peripheral blood. The priming of extracorporeal circuit was made with compatible, irradiated, leucodepleted packed red cell to avoid hypovolemic state. Citrate dextrose solution A(ACD-A), with a ratio of 1:24 to whole blood, and a bolus of heparin 50 UI/kg were used as anticoagulants. All patients, treated without sedation, were monitored by ECG, pulse oximetry and non invasive blood pressure; electrolytes panel (Na, K, Ca) and ACT (Activated coagulation time) were assessed at the beginning, 30 minutes after and then every hour during apheresis. Hypocalcemia was managed by 350 mg calcium gluconate slow infusion. We report our experience of PBSC collection in low body weight children (< 12.5 kg) treated in our Apheresis Department between January 2015 and November 2016. A total of 37 PBSC collections were performed in 17 children (8 M/9 F, median age 21 months, median weight 10.5 kg) affected by Medulloblastoma ( $n=4$ ), Germ Cell Tumor ( $n=2$ ), Neuroblastoma ( $n=8$ ), Retinoblastoma ( $n=2$ ), Brain Cancer ( $n=1$ ). Total blood volume processed ranged from 2.0 to 4.55 TBV (median 3.05) and median count of CD34+ collected was  $5.5 \times 10^6$ /kg (range: 1.5–54). All procedures were performed with a median duration time of 199 minutes (range: 114–293 min) and no serious adverse events occurred. In our experience PBSC collection is safe and feasible also in low body weight children using a tailored apheresis procedure.

**Disclosure of conflict of interest:** None.

#### P046

##### Plerixafor on demand in the first or in the second attempt of CD34 mobilization

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High-dose chemotherapy and autologous hematopoietic cell transplantation (auto-HCT) is a recommended strategy for patients with relapsed, refractory or high risk lymphoma. Mobilization failure of CD34+ cells after granulocyte colony-stimulating factor (G-CSF) with or without chemotherapy is a factor limiting patient access to this potentially curative procedure. The use of plerixafor with G-CSF may improve CD34+ cell harvest in poor mobilizing patients. We evaluated the clinical effectiveness of plerixafor and G-CSF  $\pm$  chemotherapy administered on demand in the first and second attempt of mobilization in lymphoma or myeloma patients who were eligible for auto-HCT. We evaluated data on 59 consecutive patients with Hodgkin lymphoma (17), DLBCL (17), mantle cell lymphoma (10), myeloma (8) and other lymphoma subtypes (7) who were mobilized with plerixafor between January 2011 and October 2016. Median (range) age of patients was 47 (27–69). Patients received a median of 2 (1–4) chemotherapy lines. Radiotherapy was applied in 13 patients. All patients received G-CSF (10  $\mu\text{g}/\text{kg}/\text{day}$ )  $\pm$  chemotherapy and plerixafor (240  $\mu\text{g}/\text{kg}/\text{day}$ ) on demand in the absence of increase in the number of CD34+ cells in peripheral blood above 10/ $\mu\text{L}$  on the day of the scheduled apheresis (within 20 days following the chemotherapy and after at least 4 days of G-CSF). Plerixafor was given to 36 patients in the first attempt of mobilization and to 23 patients during the second mobilization. The mobilization was considered effective if the harvest cell dose was  $2 \times 10^6$ /kg CD 34 or more. After plerixafor administration circulating CD34+ cells increased to  $>10/\mu\text{L}$  in 21 patients (58%) and in 11 patients (43%) in the first and in the second mobilization, respectively ( $P=0.26$ ). The CD34+ cell collection was performed in 52/59 patients (88%): in 32/36 (89%) patients in the first and in 20/23 patients (87%) during the second mobilization cycle. The median number of apheresis was 3 (range: 1–6), for both mobilizing cycles. The median (range) CD34 cell dose collected in the first and second cycle was 3.03 (range: 0.77–16.87)  $\times 10^6$ /kg and 1.89 (range: 18–11.53)  $\times 10^6$ /kg, respectively ( $P=0.007$ ). The harvest was successful in 27/32 patients (84%) in the first and in 10/20 patients (50%) in the second cycle ( $P=0.007$ ). Three patients (9%) who failed the collection with plerixafor in the first attempt, succeeded in the second cycle. Additional second mobilization with plerixafor was successful in five patients (25%) who failed the first mobilization. In total, 30/32 (93%) and 15/20 (75%) of patients given plerixafor in the first or in the second mobilizing cycle harvested at least a minimum CD34 cell dose for auto-HCT ( $P=0.05$ ). These results show that plerixafor administered on demand is an effective rescue strategy for poor mobilizing patients. Each mobilization cycle with plerixafor resulted in the increase of circulating CD34 cell count. Successful harvest is more frequent if plerixafor is administered in the first than in the second mobilization attempt. The evaluation of the prognostic factors for mobilizing failure with plerixafor is necessary to identify the poor mobilizers precisely.

**Disclosure of conflict of interest:** JR-J, EP-K, LT and JW: Sanofi (travel grants); MS and ZP: none; JW: lecture, honoraria

#### P047

##### Pre-clinical validation of Lovo for post-thaw DMSO depletion from cryopreserved stem cells

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Cryopreserved stem cell grafts are still widely used both in the autologous or allogeneic settings. Cryopreserved grafts can be thawed at the bedside or thawed and washed at the cell therapy laboratory. We recently reported that post-thaw washing did not impair hematopoietic engraftment, in a cohort of 2930 autologous transplanted patients receiving either unwashed or washed grafts (Calmels B *et al*, Bone Marrow Transplant. 2014). Post-thaw washing can be implemented using various methods such as manual centrifugation, automated centrifuge-based (Sepax 2, Biosafe) or spinning-membrane devices such as Lovo (Fresenius Kabi). We here report a step by step implementation of the Lovo biomedical device (BMD) for washing thawed stem cell grafts. Having defined a washing program, we aim to compare this protocol to our routine process, using the Sepax 2 BMD. We took advantage of 11 apheresis products intended for destruction and cryopreserved in 2 identical bags; after dry-thawing (PlasmaTherm, Barkey), bags were connected to the Sepax 2 or to the Lovo BMD, diluted volume to volume with +4–8°C 6% hydroxyethylstarch 130/0.4 (Voluven, Fresenius Kabi) and processed using the SmartWash program (Sepax 2) or a 2-cycles standard wash protocol on Lovo (a cycle referring to one pass through the spinning membrane). The Lovo settings were customized for this application: reduction retentate pump rate 75 mL/min, desired inlet PCV 10%, and automated volume to volume dilution. After processing, CD34 and CD45 absolute counts and viability were evaluated by single platform flow cytometry (Stem-Kit, Beckman Coulter) and DMSO was quantified by capillary zone electrophoresis (P/ACE, Beckman Coulter). Post-wash data show comparable CD34+ cell recovery, viability and effective DMSO depletion. We conclude that Lovo enables high efficiency DMSO depletion while preserving optimal CD34 viability and recovery. Comparison with Sepax 2, a widely used automated centrifuge-based device, reveals comparable efficiency. Moreover, the length of the procedure when using the Lovo does not significantly delay the process as compared to bedside thawing. We are currently evaluating Lovo for the processing of multiple bags and higher cell contents, due to its ability to concentrate large volumes of cells suspension. Post-thaw washing using automated cell processing systems have thus to be preferred over bedside thawing, since they provide multiple benefits including a short processing time, efficient DMSO and cell debris removal, precise determination of infused CD34+ cell dose, and improved cellular stability.

[P047]

	CD34 before 10 <sup>6</sup>	CD34 after 10 <sup>6</sup>	CD34 recovery	CD34 viability	CD45 after viability	DMSO before g	DMSO after g	DMSO elimination %
lovo	184	134	79%	91%	71%	12,0	0,5	96,4%
sepax	184	142	81%	93%	71%	9,8	0,4	96,3%

**Disclosure of conflict of interest:** None.

#### P048

##### Priming with G-CSF of the bone marrow grafts in related and haploidentical stem cell transplantations

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Using bone marrow (BM) as the graft source results in lower graft-versus-host disease incidence, which is particularly important in haploidentical (haplo) stem cell transplantations (SCT). Nonetheless achieving adequate CD34+ cell count might be complicated in cases of donor-recipient weight differences. Priming with G-CSF may partly solve this problem. Also there are reports of immunomodulatory effect of BM priming. In the retrospective study we have evaluate the effect

of priming on stem cell yield and the outcomes of SCT. **Patients and methods:** 50 patients with primed BM graft were matched in the ratio 1:1 to non-primed grafts. The criteria for matching were type of the donor, age of the recipient, underlying disease and disease status at the time of SCT. Priming was performed with three injections of filgrastim 5–7 mcg/kg daily for 3 days prior to BM harvesting. Median recipient age was 18 years (range: 1–58). 60% of patients received the graft from haplo donor, 40% from matched related donor (MRD). 39% had acute lymphoblastic leukemia, 38% had acute myeloid leukemia, 10% had aplastic anemia, 13% had other malignancies. 68% were classified as salvage patients. 27% received myeloablative conditioning, 73% received reduced intensity. Post-transplantation cyclophosphamide (PTCy) was used as graft-versus-host disease prophylaxis in 83% of patients. **Results:** the yield of CD34+ × 10<sup>6</sup> cells /kg of recipient weight was only non-significantly higher in the priming group: 6.0 ± 3.4 vs 5.0 ± 2.5, P=0.12. The yield of CD34+ cells per kg of donor weight was also not different: 4.3 ± 4.0 vs 4.3 ± 5.8, P=0.55. There was no difference in the incidence of primary graft failure (14% vs 20%, P=0.42). Median time to neutrophil (21 vs 23 days, P=0.02) and platelet (19 vs 23 days, P=0.05) engraftment was shorter in non-priming group. There was no differences between priming and non-priming groups in the incidence of acute grade II–IV GVHD (14% vs 4%, P=0.11), moderate and severe chronic GVHD (12% vs 11%, P=0.88), 1-year non-relapse mortality (12% vs 18%, P=0.46), relapse incidence (36% vs 38%, P=0.91), overall survival (64% vs 54%, P=0.52), event-free survival (52% vs 44%, P=0.62) and GVHD-relapse-free survival (38% vs 36%, P=0.62). **Conclusions:** Priming of the bone marrow with reported schedule did not result in higher CD34+ cell yield and was not associated with any differences in the outcomes of SCT. Nonetheless, these results should be interpreted with caution, because our study included large proportion of pediatric patients, patients with active disease and PTCy as GVHD prophylaxis, and they may not translate to the other groups of patients.

**Disclosure of conflict of interest:** None.

#### P049

##### Priming with granulocyte-colony stimulating factor preserves the contents and abundant IFN-γ production capacity of γδ T cells

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The increasing evidences indicate that removal of αβ T-cell and B-cell from grafts was efficient and reproducible in allogeneic hematopoietic stem cell transplantation (alloHSCT). γδ T cell is one of the functional subpopulations preserved by this graft manipulation and supposed to play a role in improving the transplant outcomes. Thus, comprehensive understanding the subsets and functional capacities of γδ T cells in graft becomes important. Although there is increased attention paid on this special T-lymphocyte subpopulation, the contents and cytokine production capacities of peripheral γδ T cells before and after granulocyte-colony stimulating factor (G-CSF) mobilization for alloHSCT have not been reported. Peripheral blood (PB) before G-CSF treatment, G-CSF-primed PB and bone marrow (BM) grafts were obtained from 19 healthy donors. The proportions of total γδ T cells and various γδ T-cell subsets were detected by flow cytometry. Furthermore, effects of G-CSF on the contents and cytokines production by γδ T-cell subsets were also determined. The percentages of most γδ T-cell subsets including CD27+, CD27–, Vδ1+, Vδ1+CD27+, Vδ1+CD27–, Vδ2+, Vδ2+CD27+, Vδ2–CD27–, and non-Vδ1/δ2 were preserved in the G-CSF-primed PB grafts compared with those before G-CSF mobilization. Interestingly, we found that peripheral γδ T cells and various subsets all predominantly expressed IFN-γ in response to stimulation. This abundant IFN-γ production capacity of peripheral γδ T cells were maintained after G-CSF treatment.



Biosimilars have led to significant improvements in the affordability of growth factors such as Granulocyte-colony stimulating factor (G-CSF). Data has shown similar performance and efficiency to parent drugs but concern has been raised about their use in healthy donors due to lack of data examining adverse effects in this setting. We conducted a retrospective analysis investigating mobilisation and adverse effects in 51 healthy sibling donors of adults undergoing an allogeneic haematopoietic stem cell transplant at St Bartholomew's Hospital from 2011 to 2014. Harvest data were gathered from hospital records. Adverse effects data were gathered from hospital records and telephone follow up. 58% of donors were male with a median age at harvest of 46 (13–65). All donors were mobilised using Zarzio™ Biosimilar G-CSF at a dose of 10 µg/kg/day. Median number of apheresis required was 1 (1–3). Median CD34+ cell count was  $5.6 \times 10^6$ /kg bodyweight (1.3–13.9) with  $1664 \times 10^6$  CD34+/ $\mu$ L (168–3779) in peripheral blood. The target CD34+ count ( $>5 \times 10^6$ /kg) was achieved in 59% of donors and an adequate yield ( $2\text{--}5 \times 10^6$ /kg) in 33%. In four donors (8%), the harvest was deemed to have been unsuccessful as the CD34+ count was  $<2 \times 10^6$ /kg. The patients with donor harvest yields  $<2 \times 10^6$ /kg proceeded to transplant; all four patients engrafted and one patient had mixed chimerism at Day 28 but was fully donor by day 75. Median CD3+ cell count was  $2.7 \times 10^6$ /kg bodyweight (0.8–6.4). Median days to neutrophil engraftment ( $>0.5 \times 10^9$ /L) was 16 (0–27). Median days to platelet engraftment ( $>20 \times 10^9$ /L) was 5 (0–23) with one patient never engrafting. Forty (80%) of 51 donors were contacted at a median of 24 months (1–54) post mobilisation to establish incidence of adverse effects. Three donors were uncontactable as they had moved overseas. Eight donors were not contacted to avoid distress as their sibling had died since transplant. Among contacted donors 42.5% reported side effects including bone and lower back pain controlled with analgesia, constipation and low mood. Other side effects included chest pain which was considered to be musculoskeletal in origin on day 3 of G-CSF administration associated with taking an increased dose due to patient error ( $n=1$ ) and abdominal contractions like labour while receiving G-CSF ( $n=1$ ). Three (7.5%) reported side effects lasting beyond one month post mobilisation: lower back pain lasting 2 months ( $n=1$ ), fatigue of 3 months duration ( $n=1$ ), and cough of 8 months duration ( $n=1$ ). Our data demonstrates good mobilisation using 10 µg/kg/day Zarzio™ biosimilar G-CSF without significant adverse effects at 2 years median follow up. This supports its ongoing use for the mobilisation of healthy donors. **Disclosure of conflict of interest:** SGA has received honoraria from Sandoz and grant support from Sandoz and Amgen.

#### P053

##### **Stem cell mobilization in poor mobilizers with multiple myeloma (MM) or non-Hodgkin lymphoma (NHL) before and after introduction of plerixafor: Single center comparative analysis using a cost-efficient single fixed-dose schedule**

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Collection of hematopoietic stem cells (HSC) from the peripheral blood (PB) is routinely conducted prior to high-dose chemotherapy and autologous transplantation. Despite safety and efficiency of current apheresis procedures including mobilizing chemotherapy and granulocyte colony-stimulating factor (G-CSF), there is a significant rate of mobilization failures due to different patient-dependent factors necessitating additional agents like plerixafor. While plerixafor is approved for patients with MM or NHL based on prospective studies using steady state mobilization with G-CSF +/- plerixafor, prospective studies using chemo-mobilization are lacking. Here we compared the outcome of poor mobilizer from the

pre-plerixafor era with poor mobilizers who received additional plerixafor in a real world analysis. We analyzed 50 consecutive patients with MM or NHL who were mobilized at our academic center between 2011 and 2016 and received plerixafor, because they were expected to be poor mobilizers, due to 1. low counts of CD34+ cells in PB samples prior to apheresis, 2. after a first apheresis day with insufficient yield or 3. as a rescue strategy after insufficient harvest with previous mobilizing chemotherapy (Greil C, Engelhardt M, Wäsch R. Leukemia & Lymphoma 2017, in press). We examined CD34+ cell counts in PB and in apheresis products to identify those patients who were able to collect a sufficient CD34+ cell count for transplantation after application of plerixafor. We compared these data with 100 consecutive poor mobilizers from the pre-plerixafor era, who were mobilized between 2000 and 2011 without plerixafor. The median PB CD34+/ $\mu$ L count at first apheresis was significantly higher after the first dose of plerixafor when compared to the pre-plerixafor group with 19.9 vs 9.8 ( $P < 0.001$ ). Accordingly, the median collected CD34+ cells/d ( $\times 10^6$ /kg bw) and total CD34+ cells ( $\times 10^6$ /kg bw) were significantly increased with 1.67 vs 0.88 ( $P < 0.001$ ) and 4.13 vs 2.66 ( $P < 0.001$ ), respectively. The rate of  $>2 \times 10^6$  CD34+ cells/kg bw in first apheresis (%) increased from 11% in the pre-plerixafor era group to 38% after the first dose of plerixafor in the plerixafor group. Consistently, the successful transplantation rate increased from 58% in the pre-plerixafor group to 90% in the plerixafor group. Successful stem cell mobilization could be achieved with only a single fixed-dose of plerixafor in 62% of poor mobilizers as previously reported by our group. The addition of plerixafor to chemo-mobilization in poor mobilizers with MM or NHL significantly increased PB CD34+/ $\mu$ L counts, apheresis yields and transplantation rates when compared to poor mobilizers from the pre-plerixafor era. These favorable apheresis results can be obtained using our cost-efficient, single fixed-dose plerixafor schedule in the majority of the patients leading to a 90% transplantation rate in poor mobilizer.

**Disclosure of conflict of interest:** RW received research funding, advisory and speaker's honoraria from Sanofi-Aventis.

#### P054

##### **The choice between stem cell mobilisation with G-CSF alone or with G-CSF plus chemotherapy may impact on the stem cell transplant service**

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Successful stem cell mobilisation is essential for successful autologous transplantation. G-CSF alone or G-CSF combined with chemotherapy are commonly used and differ with respect to CD34+ yield and complication rates. In a prospective audit we compared the efficacy of biosimilar G-CSF alone (G-only) or in combination with etoposide (E+G-CSF), together with the use of subsequent plerixafor for cases failing mobilisation. We included all patients who underwent stem cell mobilisation (SCM) between 01/02/15 and 01/10/2016. We aim to collect a minimum of  $2 \times 10^6$  CD34+ cell/kg. All multiple myeloma (MM) patients received G-only; all patient with germ cell tumour (GCT) received E+G-CSF, and patients with non-Hodgkin lymphoma (NHL) or Hodgkin disease (HD) received either G-only or E-G-CSF at clinician discretion. Etoposide (0.8 g/m<sup>2</sup>/day) was given on Day (D) 1 & 2 and G-CSF (5 µg/kg/day) from D3 to D13. Apheresis took place on D13 if PB CD34+ count was  $>10/\mu$ L. In the G-only regimen G-CSF was given subcutaneously at 10 µg/kg daily for 5 days and harvest was scheduled on the fifth day if PB CD34+ count was  $>10/\mu$ L. Of a total of 128 patients (81 MM, 26 NHL, 11 HD and 10 GCT; 90 and 38 received G-only and E+G-CSF, respectively. Compared with E+G-CSF, G-only

yielded a significantly lower PB CD34<sup>+</sup> cell count and PB CD34<sup>+</sup> cell percentage; median 32.4/ $\mu$ L (1.56–286) vs 95.16/ $\mu$ L (2.7–2002),  $P < 0.0001$  & median 0.08% (0.1–1.5) vs 0.72% (0.02–6.2),  $P < 0.0001$ , respectively. In G-only, 26/90 (28.8%) required 1 day of apheresis, 53/90 (58.8%) 2 days and 10/90 (11.1%) 3 days. In E+G-CSF group 28/38 (73.6%) required 1 day, 7/38 (18.4%) 2 days and 2/38 (5.2%). A median harvested CD34<sup>+</sup> cell dose of  $4.2 \times 10^6$ /kg (0–11.9) vs  $6.63 \times 10^6$ /kg (0.37–69);  $P = 0.002$  was achieved in G-only compared with the etoposide group. Thirty eight patients (42.2%) in the G-only group required plerixafor, of whom 24/38 (63.1%) needed one dose of plerixafor and 14/38 (36.8%) required two doses. In the E-G-CSF group, fewer patients required plerixafor (6/38 (15.7%);  $P = 0.003$ . Only 8% (7/90) of patients mobilised with G-only required admission during mobilisation; mostly for venous access care, compared with 34.2% (13/38) of E-G-CSF group; due to neutropenic infections, and requiring a shorter stay; median 1 day (1–2) vs 5 days (1–10)  $P = 0.017$ . Patients mobilised with G-only who underwent ASCT ( $n = 81$ ) received a median cell dose of  $2.26 \times 10^6$  /kg (1.51–4.11) compared with a cell dose of  $4.17 \times 10^6$  /kg (1.42–7.61) in E-G-CSF mobilised patients ( $n = 32$ ); ( $P < .0001$ ). In keeping with this, G-only mobilised patients received more bags of stem cells; 4 (1–11) vs 2 (1–15),  $P < 0.0001$  and needed 2 days of infusion in around 20% (16/81) compared with 12.5% (6/42) with E-G-CSF group,  $P > 0.05$ . Median time to neutrophil engraftment was 12.5 days (10–28) in the G-only group vs 11 days (9–28) in the E+G-CSF group;  $P = 0.001$ , however, there was no difference in platelet ( $> 20 \times 10^9$ /L and  $> 50 \times 10^9$ /L) engraftment between the G-only vs E-G-CSF groups: 19 days (11–41) vs 20 days (11–29) and 20 days (13–43) vs 21 days (16–44);  $P > 0.05$ , respectively. All patients engrafted by day 28 and no engraftment failure was observed. In conclusion, a biosimilar G-CSF-alone approach can be recommended as a safe option; however, rescue alternatives, for example, with plerixafor, should be available for potential failures.

**Disclosure of conflict of interest:** None.

#### P055

##### The comparison of effectiveness and safety between different biosimilar of G-CSFs in mobilization of PBSC of autologous transplantation

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High-dose chemotherapy followed by autologous peripheral blood stem cells transplantation (PBSCT) is the standard of treatment for patients with hematological malignancies. Recombinant granulocyte colony-stimulating factors (G-CSFs) are widely used alone or in combination with chemotherapy, in order to mobilize patient's stem cells (CD34<sup>+</sup>) for autologous and allogeneic peripheral blood stem cells transplantation. **Aim:** The aim of our study was to compare effectiveness and safety of different biosimilar products of filgrastim used in autologous PBSC mobilization in patients with hematological malignancies. Our retrospective analysis included 282 patients (118 women and 164 men) with median age 54 years (range: 19–77), who underwent the procedure of autologous PBSCT in years 2012–2014 in the Haematology, Blood Neoplasms, and Bone Marrow Transplantation Clinic of Medical University in Wrocław. There were three different biosimilar products of filgrastim used: Tevagrastim (Teva) in 95 patients, Nivestim (Hospira) in 92 patients and Zarzio (Sandoz) in 95 patients. 90 (32%) patients were diagnosed with plasma cell neoplasms, 145 (51%) with Hodgkin's and non-Hodgkin's lymphomas, 20 (7%) patients had acute myeloid leukemia and 27 (10%) had other hematological malignancies. Statistical analysis was conducted using STATISTICA 12 (StatSoft Polska) statistical software. For quantitative variables arithmetic means and standard deviations were calculated for the estimated parameters in the studied groups. Distribution of variables was

tested using W-Shapiro–Wilk test.  $P < 0.05$ ). There were also small variations in the number of leukapheresis necessary to obtain the minimum CD34<sup>+</sup> cell count: 1.32 in Zarzio group, 1.37 in Nivestim group and 1.66 in Tevagrastim group. However, there were no difference between biosimilar G-CSFs. The highest rate of successful mobilizations (defined as  $> 2 \times 10^6$ /kg CD34<sup>+</sup> cells collected) was observed in 88.2% patients received Zarzio, in 86.2% received Nivestim and in 84.9% patients received Tevagrastim. The safety profile was comparable between the biosimilar G-CSF and included bone pain in 30 (10%) patients and headache in 25 (9%) patients. The results are shown in Table 1. All three used biosimilar G-CSFs demonstrated similar efficacy and safety in stem cell mobilization in patients with hematological malignancies. Therefore, it seems that all the analyzed products can be used interchangeably. Presented observations should be verified with wider prospective research.

[P055]

Table 1: The efficacy of biosimilar G-CSFs in hematopoietic stem cells mobilization.

G-CSF	Tevagrastim	Nivestim	Zarzio
Number of patients	95	92	95
Actual collections	84,9%	86,2%	88,2%
Apheresis not attempted	25 (26.32%)	16 (18.19%)	21(22.58%)
Mean – No. apheresis	1,66	1,37	1,32
Peak peripheral Cd34 <sup>+</sup> in blood/mikrollitr (median)	39,58	58,45	54,39
I leukapheresis (geomean) cd34 <sup>+</sup> /kg	3,25	4,69	4,68
Total cd34 <sup>+</sup> /kg (median)	5,95	7,08	6,80
Days of administration (median)	8 (4-10)	8 (5-12)	8 (6-14)
% adverse events	10 (10,5%)	11(12%)	9 (9,4%)

**Disclosure of conflict of interest:** None.

#### P056

##### Use of G-CSF mobilized bone marrow grafts in HLA-haploidentical related bone marrow transplantation (BMT) with post-transplantation cyclophosphamide (PT-CY)

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Use of G-CSF stimulation of BMT donors might prove to be beneficial in many respects, improving TNC yield but also through immunomodulatory effect on donor T cell function and APCs1. We analyzed outcomes of 14 consecutive patients receiving bone marrow transplants from HLA-haploidentical related donors that received G-CSF stimulation prior to harvest. Fourteen patients received HLA-haploidentical BMT with PT-CY between 5/2012 and 10/2016. Five donors were siblings, 4 children, 4 mothers and 1 father. Donors received G-CSF at the dose of 10 mcg/kg BW sc. on days – 2, – 1 and 0 before BM collection. Twelve patients received non-myeloablative conditioning according to Baltimore protocol2, while two patients received myeloablative conditioning (BuCy). Along with post-transplantation cyclophosphamide, all patients received tacrolimus and MMF form day +5, as described earlier 2. Median age was 41 years (range: 19–63), 7 female and 7 male patients. Eight patients had AML, 1 CML, 4 MH and one ALL. Ten of them were in remission, while 2 MH patients were in PR, and 2 AML patients had residual disease as evident by immunophenotyping. Median number of infused TNC was  $5.17 \times 10^8$ /kg BW (range: 1.84–8.21); CD34+

cells  $1.88 \times 10^6/\text{kg}$  BW (range: 1–4.47) and CD3+ cells  $1.35 \times 10^7/\text{kg}$  BW (range: 0.37–6.04). Median follow up was 362 days (range: 26–1654). Eleven patients engrafted (79%), one patient had primary rejection, one had overt disease relapse at day +35 and one patient died in aplasia due to sepsis. Median day to neutrophil recovery ( $\text{ANC} > 0.5 \times 10^9/\text{L}$ ) was 21 (range: 15–29), median days to platelet recovery ( $\text{PLT} > 20 \times 10^9/\text{L}$ ) was 31 (range: 12–45). In all patients MMF was discontinued at D +35. Two patients developed acute GVHD in our cohort (18%), one after receiving DLI for falling chimerism at day +169. One patient (9%) developed chronic GVHD, after having received DLI due to disease progression. At the time of analysis 10 patients are evaluable; 4 patients had disease relapse/progression (40%), 6 patients are alive and in remission. One patient died due to sepsis in aplasia (accounting for 7% non-relapse mortality). One patient that rejected the graft was transplanted again from the same donor, using myeloablative conditioning and peripheral stem cells as graft source and engrafted. Overall survival median is 2.7 years, with significantly shorter survival if patient was not in complete remission at time of transplant ( $P < 0.01$ ). Even though the experience with G-CSF mobilized BM graft in the HLA-haploidentical setting with PT-CY is relatively small, in our series it has been beneficial in terms of TNC yield. Also, the incidence of acute and chronic GVHD in our patients has been low, particularly aGVHD with one case developing only after DLI. Whether the observation is the result of limited number of patients, or it reflects the immunomodulatory effect of G-CSF on BM graft as previously suggested<sup>1</sup> remains to be seen as further studies are warranted.

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**Disclosure of conflict of interest:** None.

#### P057

##### WBC and CD34+ Kinetics can predict a suboptimal mobilization of hematopoietic stem cells

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Autologous transplantation of haematopoietic stem cells (aHSCT) is usually perceived as a fully standardized and safe procedure; however, a minority of patients experience a delayed engraftment and seldom even an engraftment failure, possibly related to a poor quality of the graft. Therefore the current policy in many centers is aimed to increase the target dose of collected CD34+ cells up to an 'optimal' level of  $4 \times 10^6/\text{kg}$ . Plerixafor was introduced in the clinical practice to maximize the mobilization of HSC, in order to collect an optimal number of CD34+ cells in a limited number of collections also in poor and slow mobilisers. We carried out a retrospective analysis of our case series aimed to individuate mobilization predictors optimize the 'on demand' use of Plerixafor. We analyzed 162 patients who underwent mobilization with Cyclophosphamide (4 g/sqm) and Filgrastim 10 mcg/kg from +5 in our Unit from 2009 and 2016. Diagnosis were Multiple Myeloma (MM) 74 (45.7%), Non-Hodgkin Lymphoma (NHL) 46 (28.4%), Hodgkin Lymphoma (HDG) 14 (8.6%) and 28 (17.3%) Autoimmune Disease (MS 14.8%; SSC 2.5%). Median age (range) was 53 years (16–69); Male/Female ratio 82/80. Circulating CD34+ cell count was started at White Blood Cells (WBC) recovery, which was defined as the first day when their count exceeded  $1 \times 10^9/\text{L}$ . The primary goal was to identify at WBC recovery one or more factors predicting a

suboptimal mobilization, which was defined as the failure to exceed 40 CD34+/mL circulating cells in the day after the WBC recovery. Patients were excluded from this analysis if 1) showed a CD34+ count  $> 40/\text{mL}$  at WBC recovery (very good mobilizers) and/or 2) had received Plerixafor and/or 3) did not proceed to another CD34+ count the day after WBC recovery. Binary logistic regression was used to obtain the factors that increased the odds for an optimal mobilization. Overall 80 out of 162 (49.4%) patients were shown as very good mobilisers as their CD34+ count exceeded 40/mL at WBC recovery. On the remaining 82, 7 were excluded for the lack of a second assessment and 2 for the lack of data. Among the remaining 73 patients, the threshold of 40 CD34+/mL cells on the second day was reached by 55 (75.3%) of patients (Group A) while the remaining 18 (24.7%) failed the goal (Group B). Median (range) WBC  $\times 10^9/\text{L}$  and CD34+/mL counts in group A and B at WBC recovery were 2.01 (1–6.43) and 14.65 (0.70–39.91) and 2.84 (1–20) and 7.39 (0–33), respectively, with a statistically significant differences among group (Mann-Whitney U test with  $P = 0.01$  and  $P = 0.02$ , respectively). WBC (OR = 2.193; 95% CI: 1.197–4.019) and CD34+/mL (OR = 0.858; 95% CI: 0.77–0.955) in first day count, but not gender, disease category and time from mobilization chemotherapy to first CD34+ count, were predictors of optimal mobilization. Combining these two predictors we found that WBC/CD34+ ratio has a sensitivity of 82.4% with an AUC 83.7 in ROC analysis. Assessment of circulating WBC, CD34+ and their ratio at WBC recovery in a chemo-based mobilization strategy can predict sub-optimal mobilization of HSC and support the decision of adding Plerixafor. These data will be prospectively validated in a broader set of patients.

**Disclosure of conflict of interest:** None.

## Cell therapy/Cellular Therapy

#### P058

##### A new method to generate mature (leukemia-derived) dendritic cells that improve antileukemic T-cell reactivity from mononuclear cells or whole blood from healthy volunteers or patients with AML

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Dendritic cells (DC) and 'leukemia-derived DC' (DCleu) are potent stimulators of various immunoreactive cells and play a pivotal role in the (re-)activation of the immune system. We established a new protocol, containing GM-CSF, IL-4, Picibanil and PGE1 ('Pici2'), to generate DC/DCleu *ex vivo* from mononuclear cells ('MNC-DC') or directly from whole blood (to simulate *in vivo* conditions, 'WB-DC') from AML-patients. Further, we analysed the DC-generating-potential of the IMMUNOMODULATORY KIT-M containing GM-CSF and PGE1. Results with regard to DC-, T-cell-subtypes and mediation of antileukemic activity were compared to an established DC-generating protocol ('Pici1' containing GM-CSF, IL-4, Picibanil, PGE2) or to a culture without cytokines. (1) DC-generation: healthy; 'MNC/WB-DC': We generated comparable DC-proportions with 'Pici2' and the standard protocol 'Pici1'. With 'Pici2' we significantly (p65% of cases. Further, we generated higher amounts of DCleu with the two PGE1 containing

'cocktails' (DCleu: range: 3.4%–13.6%) in comparison to controls without cytokines. (2) Mature DC: Adding PGE1 to the 'DC-generating-cocktails' increased the proportions of mature DC (CD197+) in comparison to 'Pici1' in AML-samples. This effect, however, did not occur in healthy-samples. (3) T-cell-subtypes: T-cell-stimulation with DC, generated with PGE1 containing 'cocktails,' positively influenced the composition of T-cell-subsets, characterised by higher proportions of proliferating (CD71+), non-naïve (CD45RO+), central-(memory) (CD45RO+CCR7+) and effector-(memory)T-cells (CD45RO+CCR7-). (4) Antileukemic activity: T-cells stimulated with DC, generated with 'Kit-M' and 'Pici2' highly improved antileukemic activity ('blast lysis') in >70% of cases. We developed a new DC/DCleu generating protocol and demonstrated that PGE1 is suitable for generating DC/DCleu in sufficient amounts. These cells reliably (re-)activate immunoreactive cells and improve the overall *ex vivo* antileukemic activity. *In vivo* trials (animals and/or humans) have to be performed to study potential effects of PGE1 in the mediation of antileukemic reactions *in vivo* in patients with AML.

**Disclosure of conflict of interest:** None.

#### P059

##### **A standardized and characterized clinical grade human platelet lysate for optimized expansion of human bone marrow mesenchymal stem cells**

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Human platelet lysate (hPL) is rich in growth factors (GF) and nutritive elements and represents a powerful xeno-free alternative to fetal bovine serum (FBS) notably for mesenchymal stem cell (hMSC) proliferation. However, there is a large variability in hPL preparations (various sources, use of different and non-standardized production protocols, with variable and limited number of donors), resulting in discrepancies in product quality, low management of product safety and poor batch-to-batch standardization. We describe here the development and the characterization of a standardized hPL prepared from outdated transfusional grade screened normal human donor platelet concentrates (PCs), manufactured on an industrial scale (batch size of 250 donors) and following a highly qualified process (clean room, trained operators, validated aseptic filtration). PCs were frozen at -80°C and thawed at +4°C to lyse platelets. Cell debris were removed by centrifugation and the supernatant (hPL) was recovered. Clinical grade 10L batches of aseptic filtered hPL were characterized. First, we showed that hPL prepared from a limited number of donors displayed a variability in terms of GF contents. On the contrary, we observed a robust standardization between industrial batches of hPL (250 donors) in terms of GF contents (bFGF, EGF, VEGF, PDGF-AB, TGF-beta1 and IGF-1), biochemical analyses (total proteins, albumin, fibrinogen, vitamins and iron) and efficacy on bone marrow (BM)-hMSC proliferation. Secondly, we compared expansion and functional characteristics of BM-hMSCs grown in clinical grade hPL vs MSC-screened FBS batches. We showed a reproducible increase in cell growth kinetics using hPL, a maintenance of BM-hMSC clonogenic potential and membrane marker expression (with however a strong overexpression of CD90). We observed a similar adipogenic and osteogenic differentiation potential and finally that immunosuppressive properties of BM-hMSCs (inhibition of T-cell proliferation) cultivated in parallel in both conditions also remained identical. Finally, we demonstrated the stability over time of hPL stored at -80°C and -20°C. In conclusion, we demonstrated the feasibility to

use a standardized, characterized, efficient and clinical grade hPL for research and cell therapy applications.

**Disclosure of conflict of interest:** SV, SE, LC, PB, TB, AL, FG and BD are employees of Macopharma.

#### P060

##### **Previously published**

#### P061

##### **Alpha/beta T cell depleted donor lymphocyte infusion**

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The main objective of this project is to improve a safe and efficient new Donor Lymphocyte Infusion (DLI) with depletion of  $\alpha\beta$ + T cells which cause graft versus host disease (GVHD), and enrichment of anti-leukemic  $\gamma\delta$ + T cells, NK cells and dendritic cells to build an effective and permanent anti-tumor effects for patients relapsed hematological cancers after allogeneic hematopoietic stem cell (HSC) transplantation who have blasts and mixed chimerism. This study is conducted with collaboration of Erciyes University Pediatric and Adult HSCT units, and Bahcesehir University, Medical Park Hospital Pediatric HSCT unit. The TCR  $\alpha\beta$ + T cell depleted DLI product that is used in the study was collected and separated at Erciyes University Apheresis Unit. The cell contents obtained for TCR  $\alpha\beta$ + T cell depleted DLI used for patients were CD3 cells were reduced to  $1.4\text{--}23.5 \times 10^6$  cells/kg,  $\gamma\delta$ + T cells were reduced to  $2.58\text{--}23.48 \times 10^6$  cells/kg,  $\alpha\beta$ + T cells were reduced by 99.99%, and were obtained at 5–4100 cells/kg. A total of 10 patients (4 female, 6 male) were included in the study, consisting of an adult and 9 children. Nine patients had hematological malignancies. Five patients were referred for ALL, three for AML, one for MDS and one for Griscelli Syndrome. Efficiency: The clinical response to the  $\alpha\beta$ + T cell depleted DLI treatment was achieved in 7/10 patients (70%). In these patients, although the increase of chimerism was limited in 2 patients, no recurrence was occurred. One of the two patients who previously responded to the treatment but experience of decreasing chimerism had relapsed after 2 months, and 9 months later. One of these two patients died after relapse. The other was managed by the second transplant. The most important objective of this study was to show that  $\alpha\beta$ + T cell depleted DLI treatment is reliable. None of the patient showed severe GVHD except one patient with mild grade II GVHD. Despite the presence of severe GVHD after HSCT in two patients, reactivation for GVHD was not observed after treatment with  $\alpha\beta$ + T cell depleted DLI. None of the patients had a bone marrow aplasia. As a result,  $\alpha\beta$ + T cell depleted DLI treatment seems to be highly safe, and effective in selected patients.

**Disclosure of conflict of interest:** None.

#### P062

##### **Alpha/Beta depletion as a strategy after hematopoietic stem cell transplantation to treat graft failure**

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Hematopoietic Stem Cell Transplantation (HSCT) is associated with several potentially lethal complications; for example, relapse of the malignant disease, graft rejection, infectious complications and Graft Versus Host Disease (GVHD). Higher levels of CD3+ cells in the graft have clearly been associated with increased risk of GVHD, but also superior GVL effect and less infectious complications. To tackle post-transplant complications such as graft failure and relapse, donor lymphocyte infusion (DLI) have successfully been used for decades but with an associated risk of GVHD. To decrease the risk of GVHD but still use facilitating cells in the cell product we performed

$\alpha\beta$  depletion of grafts for use as stem cell booster after allogeneic HSCT to treat infections or poor immune reconstitution. In this study, 11 patients were infused post-HSCT with  $\alpha\beta$  T-cell depleted grafts. The indication for infusion of  $\alpha\beta$  T-cell depleted graft in all patients was poor immune reconstitution with associated infectious complications. For all 11 patients, the original HSCT donor was used for the  $\alpha\beta$  T-cell depleted boost. To characterize the  $\alpha\beta$ -depleted stem cell grafts, samples were stained for various cellular subsets and analyzed by flow cytometry. We could show a median Log depletion of  $\alpha\beta$  cells of 4.1 and a median Yield of  $\gamma\delta$  T-cells (%) of 61.4. The median CD34+ cell dose ( $\times 10^6$ /kg) was 5.5. All 11 patients were alive 3 months after infusion. After 1 year only one patient succumbed. Despite that the majority of patients suffered from aGVHD grade 2 or 3 before infusion of  $\alpha\beta$  T-cell depleted graft none showed increased symptoms afterwards. In more than 70 % of the patients there was an increase in granulocytes, thrombocytes and white blood cells 3 months after infusion. In conclusion, we describe the use of  $\alpha\beta$  T-cell depleted grafts as stem cell booster in 11 patients suffering infectious complications due to graft failure after HSCT with encouraging results. **Disclosure of conflict of interest:** None.

#### P063

##### Previously published

#### P064

##### Bone marrow graft composition and engraftment after HPCT in children

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Delayed engraftment or graft failure still remains a concern in bone marrow transplantation (BMT). Graft composition may predict engraftment after infusion. This study aims to determine which quality control parameters used for the characterization of bone marrow grafts are the most predictive in order to minimize the risk of engraftment delay or graft failure. We conducted a multicenter retrospective study in pediatric patients who underwent first allogeneic BMT at two centers in Barcelona (Catalonia, Spain) between 2011 and 2015. Quantitative variables considered for the study were: Total Nucleated Cells (TNC), Mononucleated Cells (MNC), CD34+ cells, CD3+ cells and Granulocyte-Monocyte (GM) colonies enumeration. Qualitative variables considered for the study were viability assessed by flow cytometry and clonogenic efficiency of the CD34+ cells (CLONEgm) which is the ratio between GM colonies and CD34+ cells. 85 patients were included (median age (range) were 7 years old (0–16)). The median TNC(range) was 4.26E8/kg (0.51–29.18E8/kg) and 1.1E8/kg (0.48–5.88E8/kg) for mononuclear cells (MNC). On the other hand, the median (range) CD34+ cell dose was 5.03E6/kg (0.85–24.91E6/kg) and T-cell dose (CD3+) was 0.41E8/kg (0.10–44.86E8/kg). The median (range) colony-forming unit granulocyte macrophage (GM/kg) dose was 4.35E5/kg (0.33–48.62E5/kg). The median (range) of CD34+ cell viability, was 95% (63–99%) and the median(range) of the clonogenic potential of CD34+ cells (CLONEgm) was 10.35% (1.22–76.17%). The median (range) of engraftment was 22(10–35) days for neutrophils and 20(11–38) days for platelets. 1 patient was considered as primary graft failure. In the univariate analysis, CD34+ ( $P=.049$ ) and MNC ( $P=.045$ ) cell dose predicted a faster neutrophil engraftment and female donor a slower neutrophil and platelet engraftment ( $P=.012$  and  $P=.040$ ). Cell viability also correlated to a better platelet engraftment ( $P=.030$ ). In the multivariate analysis we

observed a trend for a faster neutrophil recovery according CD34+ cells infused. Again, female donor was associated with slower engraftment. In order to establish a safety threshold, we did a quartile analysis of CD34 dose and found 3.18E6/kg (quartile 25) discriminates a faster neutrophil engraftment [median 25 days vs 21 days for those with higher CD34+ cells ( $P=.026$ )]. In conclusion, we found an association between MNC and CD34+ cell dose and time to engraft, and established a safety threshold of 3.18E6 CD34+/kg. Also, BM grafts from female donors were associated with slower engraftment. No other qualitative parameters were predictive of engraftment. **Disclosure of conflict of interest:** None.

#### P065

##### Previously published

#### P066

##### Cellular immunotherapy with multiple infusions of ex vivo expanded haploidentical natural killer cells after autologous transplantation for patients with plasma cell myeloma

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Plasma cell myeloma (PCM) is currently treated with chemotherapy and autologous stem cell transplantation (ASCT) but relapse rates remain high. Adoptive transfer of mature haploidentical natural killer (NK) cells is a promising approach to provide PCM patients with highly immunocompetent effector cells with anti-myeloma function early post transplantation. Here we report on the current clinical Phase I/II trial of multiple preemptive infusions of good manufacturing practice (GMP) expanded NK cells to PCM patients (clinicaltrials.gov NCT01040026). Ten PCM patients were recruited (seven males, three females, median age: 59y). All patients received four cycles of VTD chemotherapy (reaching CR: 4 $\times$ , VGPR: 5 $\times$  and PR: 1 $\times$ ) before high dose therapy with melphalan 200 mg/m<sup>2</sup> and ASCT. After successful stem cell mobilization and cryopreservation of patients' stem cells after the third VTD cycle, NK cells from haploidentical family donors were purified from unstimulated leukapheresis by T cell depletion and NK cell selection using CliniMACS. Highly pure NK cells (mean: 4.8 $\times 10^8$  cells) were obtained with a minimal T cell contamination corresponding to a 6.1 log T cell depletion. NK cells were expanded *ex vivo* for 19 days in GMP-medium containing autologous irradiated feeder and interleukin-2 and -15. NK cell numbers increased 54-fold (range: 38–76-fold). In three NK cell products T cell contents were 11 $\times 10^5$  cells/kg body weight (BW: 10 $\times$  above limit of clinical trial) and were successfully reduced by 2 $^\circ$  CD3-depletion to 0.3 $\times 10^5$  cells/kg BW. NK cell products were cryopreserved in escalating doses (1.3 $\times 10^6$ , 1.3 $\times 10^7$  and rest as multiple doses of maximal 1.0 $\times 10^8$  cells/kg BW). The PCM patients received 65–460 $\times 10^8$  expanded NK cells (median: 3.8 $\times 10^8$  cells/kg BW, range: 0.9–5.7 $\times 10^8$  cells/kg BW) as 3–8 infusions (median, 6 DLIs). The NK-DLIs were administered between day 2 and 21 after ASCT and were well tolerated without any acute adverse events. No signs of acute or chronic graft-versus-host disease were observed in any of the patients after a total of 57 NK-DLIs. Engraftment occurred between days 13–24 (median: 16 days). Infused donor NK cells were monitored by short-tandem repeats PCR. Donor NK cells were detected in peripheral blood one and 20 h post infusion (% donor NK of enriched blood NK cells: mean: 30%, range: 9–90%, and mean: 17%, range: 0–33%, respectively) indicating significant NK cell survival in recipients in the absence of IL-2 support *in vivo*. Clinical responses at last follow-up compared to a retrospective cohort of matched control patients will be presented. These results demonstrate the feasibility of large-scale GMP expansion and safety and

tolerability of multiple high-dose infusions of human NK cells as immunotherapy after stem cell transplantation for PCM.  
**Disclosure of conflict of interest:** None.

#### P067

##### Clinical scale production of leukemia specific t-cells from non-transplantable cord blood units

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Immunotherapy with third-party leukemia-specific T cells (leuk-STs) represents an attractive approach for acute leukemia (AL) patients lacking a fully matched donor or relapsing after allogeneic hematopoietic cell transplantation (HCT). Its application however, is limited by the demand for high numbers of antigen presenting cells (APCs), capable to produce clinically relevant numbers of leuk-STs. Low volume, non-transplantable cord blood units (CBUs) could theoretically serve as an easily accessible source to generate high numbers of dendritic cells (DCs) and subsequently leuk-STs, providing also the advantage of reduced alloreactivity, even in cases of partial matching. Our goal was to generate clinically relevant doses of leuk-STs targeting AL-related antigens, the Wilms tumor protein (WT1) and the Preferentially Expressed Antigen in Melanoma (PRAME), through the exploitation of non-transplantable CBUs. To generate DCs, immunomagnetically enriched CD34+ cells from CBUs  $\leq 70$  mL were cultured in G-rex devices in the presence of SCF, GM-CSF and IL-4. DCs matured by Toll-Like Receptor ligand 3 and 7/8 were immunophenotypically characterized by flow cytometry (FCM). Secreted cytokines were measured with ELISA. Matured DCs were activated with a peptide-mix of WT1 and PRAME and used as APCs to repeatedly stimulate naive T-cells (derived from the CD34-fraction of the same CBU). The phenotype and the specificity of generated leuk-STs were determined by FCM and IFN- $\gamma$ /ELISpot, respectively. Starting from mean  $4.2 \times 10^5 \pm 1.1 \times 10^5$  CD34+ cells, from 4 non-usable CBUs, we generated  $3.3 \times 10^9$  (range:  $1.9 - 5.7 \times 10^9$ ) myeloid DCs (CD33+/CD11c+:  $76.8 \pm 5.5\%$ ) in 35 days (fold change  $\sim 11,000$ ). The produced cells highly expressed maturation markers (CD11c+/CD40+:  $79 \pm 12\%$ ; CD11c+/HLA-DR+:  $78 \pm 10\%$ ) and secreted high levels of Th1-cytokines (IL-12:  $224 \pm 185$  pg/mL; IL-6:  $1.9 \pm 0.1 \times 10^5$  pg/mL, TNF- $\alpha$ :  $5268 \pm 1316$  pg/mL) and low levels of the Th2-cytokine, IL-10. The average number of CD34- cell-derived leuk-STs after 4 week-culture was  $7.5 \pm 3.4 \times 10^7$  ( $\sim 2$  logs above clinical doses). The produced cells were enriched in CD3+ polyclonal cells ( $80 \pm 7\%$ ), comprising of CD4+ ( $28 \pm 10\%$ ) and predominantly CD8+ cells ( $52 \pm 17\%$ ), expressing effector memory (CD45RA- /CD62L-:  $52.8 \pm 5\%$ ) and effector memory RA markers (TEMRA: CD45RA+/CD62L-:  $46 \pm 4\%$ ), while containing insignificant numbers of CD4+/CD25+ cells ( $1 \pm 0.5\%$ ). Specificity was seen after the second stimulation at the earliest and was increasing after each stimulation [mean spot forming cells (SFC)/ $2 \times 10^5$  cells at second, third, fourth stimulation:  $106 \pm 33$ ;  $422 \pm 111$ ;  $1335 \pm 314$ ; respectively]. In particular, produced cells were highly specific for both targeted antigens (PRAME:  $1019 \pm 275$ , WT1:  $316 \pm 55$ ), while they expressed low the Programmed cell death protein-1 (CD3+/PD-1+:  $9 \pm 4\%$ ), implicating absence of cell exhaustion after repeated stimulations. We report a paradigm of 'circular economy' in science, by the exploitation of non-usable CBUs, towards scalable generation of CB-CD34+-cell-derived DCs and CB-CD34-cell-derived leuk-STs from the same CBU and establishment of leuk-STs banks. Whether similarly produced leuk-STs could significantly advance the treatment of AL or leukemic relapse after HCT, will be ultimately determined *in vivo*.

**Disclosure of conflict of interest:** None.

#### P068

##### Comparison of two different methods to generate anti-fungal-specific T-cells under pre-clinical-scale conditions

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Invasive infections with *Aspergillus fumigatus* constitute a major cause of morbidity and mortality in immunocompromised patients after haematopoietic stem cell transplantation. Although adoptive immunotherapies against viral pathogens are already in phase I/II trials, clinical-grade methods for the generation of *Aspergillus*-specific T-cells (Asp-T-cells) from healthy transplant donors or even related or unrelated third-party donors are still under development. In this study, two different strategies Interferon-gamma (IFN-g) Cytokine Capture System (CCS) vs short-term *in vitro* expansion (STE) were performed from the same healthy volunteers in order to evaluate the most suitable approaches for the in-time generation of clinical applicable Asp-T-cells. PBMCs from leukapheresis of healthy donors ( $n=6$ ) were first prepared in Hannover for the IFN-g-CCS and then sent to Vienna to prepare the STE. All donors belong to the alloCELL registry (www.allocell.com) of Hannover Medical School and the frequency of Asp-T-cells was pretested by high-throughput IFN-g ELISpot assay. For the IFN-g-CCS,  $1 \times 10^7$  cells were stimulated for 16 h with GMP-conform *Aspergillus* lysate followed by magnetic selection of IFN-g-producing T cells. Cells were characterized for phenotype and function by multicolour flow cytometry. For the STE,  $20 \times 10^7$  cells were cultured in G-Rex devices and stimulated for 12 days with either the *aspergillus* lysate alone or with pooled overlapping pepmixes (CatB, Crf1, f22, Gel1, pmp20, SHMT and SOT) and IL-15. To further characterize the final cell products, multicolour flow cytometry, IFN-g ELISpot and IFN-g/granzyme B FluoroSpot analyses were performed. IFN-g-CCS: Frequency of IFN-g positive ASP-T-cells pre-magnetic enrichment ranged between 0.07 and 0.16%. Recently we defined T-cell donors as eligible if  $\geq 0.03\%$  specific IFN-g+ T cells are detectable. The purity of Asp-T-cells among CD3+ cells, obtained from three donors after magnetic selection was in mean  $64\% \pm 3$  (range: 58–69%). The absolute number of selected IFN-g+ CD3+ T-cells was  $706 \pm 194$ . This could be approximately multiplied by a factor of 100, if  $> 1 \times 10^9$  PBMCs are used for the generation of clinically applicable T cells using the CCS and the Prodigy device. STE: After 12 days, Asp-T-cells ( $n=3$ ) showed highly specific activity against the lysate (in mean  $1339 \pm 79$  spot forming colonies (SFC)/105 cells) and pooled pepmixes (in mean  $892 \pm 276$  SFC/105 cells). In both methods (lysate vs pooled pepmixes), predominantly CD4+ T-cells were expanded ( $84\% \pm 2.3$  vs  $82\% \pm 5.3$  of CD3+) compared to CD8+ T-cells ( $12.6\% \pm 2.9$  vs  $14.7\% \pm 5.3$ ). Interestingly, whereas after STE, CD4+ T-cells include mainly central memory T-cells (mean 40%; CD62L+CD45RA-), CD8+ T-cells include mainly effector memory T-cells (27%; CD62L-CD45RA-). Generated T cells were highly functional and cytotoxic as determined by the secretion of effector molecules granzyme B and IFN-g. Based on the purity of up to 69% after the IFN-g-CCS and the high number of SFC received after STE with lysate and pepmixes, both methods seem to be suitable for clinical-scale productions. For patients who are in need for high Asp-T-cell numbers the application of first in-time CCS-purified Asp-T-cells followed by the administration of STE cells might be a promising way to boost antigen-specific T-cell response.

**Disclosure of conflict of interest:** None.

P069

**Complete computerization of cell therapy product files ('zero paper') in the Qap 10 software**

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The computerized management of Cell Therapy Products (CTP) is an obligation for processing laboratories to meet regulatory requirements. The software used is often independent of institutional systems in view of the specificity of cellular therapies and do not always allow the implementation of the 'zero paper' policies that are being put in place. We report here our experience with the Qap10 software (Quality Assurance Partner) developed by the company Simédia (www.qap10.com) in open source (MIT license) allowing the management of fully computerized CTP files. The Qap10 software has been developed to ensure the traceability of CTP for both preparation and quality control by combining the product preparation environment (personnel, premises, reagents, consumables, equipment). Initially, with the help of the company Simédia, we parameterized the software in accordance with our procedures for the preparation and quality control of CTP. We built a file that we printed out for archiving on paper. It soon seemed necessary to reverse this mode of operation to add to the software the documents papers to obtain a file completely computerized and to avoid paper archiving. The close collaboration between the cell therapy laboratory staff, the software referent within the information system department of the Amiens hospital and the company Simédia enabled: Set up a document backup server sufficiently proportionate in memory. Have Simédia carry out the necessary developments so that all documents can be integrated into the software, Set up a coherent working circuit, Organize the registration of documents, Put in place a rigorous verification of the mandatory elements of the file. The reflection on the computer file made it possible to evolve the software to widen its use to all documents of management of the laboratory: Maintenance of equipment, Control of premises, Housekeeping, Staff training, Quality control of automatons, Reagents and consumables, Process, Reception, distribution. Rigorous formalization was mandatory to ensure that the record was organized in a uniform manner. An intermediate paper record is still necessary for a period of about 1 month: from the programming of the graft to the final validity of the injected product. This folder consists only of transient elements that cannot be integrated into the Qap 10 software immediately. The transition from the paper file to a computer file took place in several stages, calling into question our functioning. The difficulties of this implementation are of several natures: The heterogeneity of the documents components a cell therapy product file, The impossibility of benefiting from an interface between all computer software used on the hospital, The psychological barrier prompting us to keep a paper copy, Work habits, The guarantee of computer backup quality as well as its verification. But the complete computerization of the CTP file has the following advantages: Easy and secure accessibility of information, Resolution problems archiving paper files, A single backup media folder.

**Disclosure of conflict of interest:** None.

P070

**Conditioned media from allogenic mesenchymal stem cell culture (MSC-CM) enhances wound healing in an allogenic 3D skin model**

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Migration of the epidermal layer towards the wound centre is an important step in the healing process. Full thickness *in vitro* skin models can be used to investigate epidermal migration towards an injury site. Since wound healing therapies often require allogenic transplantation of primary keratinocytes, an allogenic 3D skin model was developed to investigate epidermal migration. The effect of mesenchymal stem cell conditioned media (MSC-CM) was assessed for wound healing using this *in vitro* human 3D skin model. Human MSCs were derived from human hip joints, and characterised using standard protocols. At 80% confluence, MSC secretions were collected in serum free medium and referred to as MSC-CM which were then analysed for protein content using ELISA. Fully humanised allogenic 3D skin models were developed ( $n=3$ ) and a 3 mm punch was induced into each model followed by daily treatment with MSC-CM to investigate the migration of the epidermal layer towards the punch centre over the dermal layer at different time points (1 week, 2 weeks, and 4 weeks). Intact and wounded models were characterised structurally by haematoxylin/eosin (H&E) staining and immunofluorescence (IF) was used to validate the dermal and epidermal biomarkers such as collagen 3 (Col3), cytokeratin 14 (K14), keratin 10 (K10), loricrin and involucrin. MSCs were characterised as stipulated by the International Society for Cell Therapy, that is, fibroblast like cells with the ability to differentiate into tri-lineages (adipocyte, chondroblast and osteoblast). Phenotypically, over 95% of the cells were able to express phenotypic markers for variant stem cells such as CD73, CD90 and CD105. Over 95% of the cells were negative for the expression of CD14, CD19, CD34, CD45 and HLA-DR ( $P=0.025$ ). MSC-CM contained different concentrations of a variety of growth factors such as keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), stromal-derived factor-1 (SDF-1) and macrophage stimulating protein-1 (MSP-1). H&E staining showed that the models had distinct dermal and epidermal layers similar to that of real skin. Additionally, IF showed that the models expressed dermal and epidermal biomarkers, for example, Col3, K14, K10, loricrin and involucrin. After treatment with MSC-CM, the epidermal multilayers of the punched models started to migrate towards the punch centre and covered the whole punched area after 4 weeks of treatment with recovered expression of the epidermal biomarkers, for example, K14, K10, loricrin and involucrin. A fully humanised allogenic 3D skin model is a useful tool to mimic the *in vivo* environment and evaluate the wound healing process. It could also be used as a screening method to test candidate wound healing drugs. Allogenic keratinocytes could be used as a cellular sheet to cover the wound area with the ability to migrate towards the wound centre and promote wound healing. A possible explanation for promoting epidermal migration at the injury site is that MSC-CM contains cytokines which accelerate cell migration such as KGF, SDF-1 and MSP-1, in addition to other cytokines which promote both migration and proliferation of epidermal cells, for example, HGF and PDGF.

**Disclosure of conflict of interest:** None.

P071

**Cryopreservation and thawing of hematopoietic stem cells CD34 induced apoptosis through caspases pathway activation**

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Cryopreservation is the only reliable form of long-term storage of viable hematopoietic stem cells (HSC). This process is increasingly used to conserve HSC CD34+, between collection and infusion through an autologous HSC transplantation. Cell damage inescapably occurs during both the freezing and the thawing process. In order to estimate HSC injury, currently

before each freezing and after each thawing, a quality control is performed including a minima: (i) CD34+ quantification; (ii) estimation of the percentage of HSC CD34+ viability, via 7-aminoactinomycin-D (7-AAD) staining and (iii) evaluation of HSC functional ability to form colony 'CFU-GM' (Colony Forming Unit—Granulocyte Macrophage). Apoptosis, or programmed cell death, involves complex pathways in part the path Fas-Fas ligand (FasL), mitochondrial components and caspase enzymes. The involvement of apoptosis dependent on caspases activation pathway in HSC CD34+ after thawing remains unknown. Here, we assess the extent of apoptosis caspase-dependent before and after cryoconservation of HSC CD34+, using a Fluorescent labeled Inhibitor of caspases 'FLICA.' We tested the induction of apoptosis caspase-dependent, before and after HSC CD34+ cryoconservation from patients with different hematological malignances: multiple myeloma ( $n=21$ ), Lymphoma ( $n=19$ ). Caspases pathway activation status was evaluated by flow cytometry, using a Fluorescent Labelled Inhibitor of Caspases 'FLICA' staining test, in HSC CD34+, lymphocytes CD3+, monocytes CD14+ and natural killer cells CD56+. In order to assess cell viability, cells were stained in parallel with 7-AAD. We determined positive cells %, that is, showing caspase activation in viable cells (FLICA+ cells), before and after cryoconservation. Caspase pathway activation level was then correlated with HSC functional ability to form colony 'CFU-GM,' and day's number of clinical aplasia. In our cohort, we showed a significant caspases pathway activation, with 18.9% CD34+ FLICA+ cells after thawing, compared with the 2.4% described in fresh CD34+ cells ( $P < 0.0001$ ). Moreover, caspases pathway was significantly activated in thawing CD3+, CD56+ and CD14+ cells: FLICA+ cells % in thawing cells were, respectively, 16.8%, 31.1% and 6.2% vs 3%, 9.7% and  $< 1\%$  in fresh cells. We also report a significant increase of apoptosis caspase-dependent in lymphoma patients (22.6% of CD34+ FLICA+) in comparison to myeloma patients studied (18.6% of CD34+ FLICA+) ( $P < 0.0001$ ). In contrast, no correlation has been established between observed caspases pathway activation and HSC CD34+ capacity to form CFU-GM, or still day's number of clinical aplasia. Our results show substantial cell death, induced by the increase in caspases pathway activation, secondary to the thawing process, and across all study cell types. This advance of apoptosis caspase-dependent may affect the immune response quality during recipient aplasia, without detecting a clinical impact. Moreover, caspases pathway activation through CD3+ and CD56+ subpopulations could modify the therapeutic result of donor lymphocytes infusion DLI, though yet untested. Thawing process in autologous graft induces apoptosis caspase-dependent in all apheresis product cells, particularly in HSC CD34+, without clinical impact in graft fate.

**Disclosure of conflict of interest:** None.

#### P072

##### **Donor-derived NK cell infusion combined with HLA haploidentical blood stem cell transplantation to decrease leukemia relapse for high risk acute myeloid leukemia patients**

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HLA haploidentical blood stem cell transplantation have solved the donor deficiency for patient who need to treat by transplantation. The high relapse of leukemia especially for high risk patient post transplantation affect the outcome of haploidentical stem cell transplantation. Natural Killer (NK) cells are part of the innate immune system and play a scavenger role to detect targets marked by 'missing self' induced by viral infection or malignant transformation. Infusion NK cells into recipient prior to stem cell transplantation could decrease the GVHD in mouse bone marrow

transplantation model. In an effort to decrease the leukemia relapse and GVHD after haploidentical stem cell transplantation for high risk acute myeloid leukemia patients, we evaluated the addition of donor-derived NK killer cells before haploidentical stem cell transplantation in high risk acute myeloid leukemia patient. Here we report interim results for five patients enrolled last year. Five high risk acute patients received haploidentical stem cell transplantation combined with donor-derived NK cells infusion. All patients received an FBCA conditioning regimen, which consisted of fludarabine (25 mg/m<sup>2</sup>/day, intravenous) on days -9 to -5, busulfan (3.2 mg/kg/day, intravenous) on days -8 to -5, cyclophosphamide (60 mg/kg/day, intravenous) on days -3 to -2 and rabbit antilymphocyte globulin (ATG 2.5 mg/kg/day, intravenous) on days -5 to -1. Donor-derived NK cells were infused into patient prior to stem cell transplantation. GvHD prophylaxis was a combination of cyclosporine A (CsA) and short term methotrexate. Five high risk patients with AML M5 CR2, 1 patient with AML M5 NR, 1 patient with AML M0 CR2 and 1 patient with AML M2 CR2) enrolled from Jan 2015 to Nov. 2015; The donors are parents and sibling. HLA were mismatched between donor and patients. Median CD34+ dose infused was 5.06/kg (range: 2.3–106/kg) and the NK cell dose infused was  $1 \times 10^8$ /kg (0.8– $1.2 \times 10^8$ /kg). All five patients got hematology recovery and achieved hematology CR. Only one patient occurred grade II aGVHD post transplantation and controlled by methylprednisolone. At a median time of 12 months (range: 9–16 months) post peripheral blood stem cell transplantation, the incidence of acute GVHD grade II is 20% (1/5). No chronic GVHD observed. Four patients are still CR and survival with event free survival with median 1 year follow up. One patient with AML M5 who had not achieved remission before transplant relapsed after 6 months and got CR with second NK infusion and still survival. NK infusion prior to transplantation was found to be safe and feasible. There was no increase acute GVHD or chronic GVHD risk. There was a trend towards increased 1-year survival for high risk leukemia patient. The potential benefit on overall survival remains to be further evaluated with additional patient enrollment and longer follow up. However, given the favorable safety profile of NK cells, future strategies to enhance efficacy such as repeat dosing or modification of NK cells are worth potential exploration.

**Disclosure of conflict of interest:** None.

#### P073

##### **Donor lymphocyte infusion after haploidentical stem cell transplantation with post-transplant CY**

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Donor lymphocyte infusion (DLI) is a therapeutic option in the treatment or prevention of relapse after allogeneic stem cell transplantation (alloHSCT). Of note, the risk of graft-versus-host disease (GVHD) associated with the graft-versus-tumor (GVT) effect may be influenced by the level of HLA disparity between donor and recipient. Data on use of DLI after unmanipulated haploidentical HSCT (haploHSCT) with post-transplant cyclophosphamide (PT-Cy) are still currently limited. We report 7 patients (pts) receiving DLI between 2014 and 2016 for prevention or treatment of relapse after haploHSCT. Seven pts were given 11 haploDLI doses, as treatment for relapsed disease ( $n=4$ ) or as preventive therapy of relapse for high risk disease ( $n=3$ ). Four pts had acute myeloid leukemia (AML), 1 had acute lymphoblastic leukemia and 2 lymphomas -1 Hodgkin (HL) and 1 non-Hodgkin DLBCL. 1 pt had intermediate risk disease features, 1 adverse risk and 5 pts had refractory disease at time of haploHSCT. 4 pts had a previous HSCT (2 allogeneic and 2 autologous). 6 of the 7 pts received a RIC regimen and the source of stem cells was peripheral blood

(n=5) and bone marrow (n=2). GVHD prophylaxis was cyclosporine and mycophenolate mofetil (MMF), ATG and PT-Cy. Median follow-up after haploHSCT was 27 (range: 6–42) months. Median time to neutrophil and platelet (>50G/L) recovery were 16 and 24 days, respectively. After haploHSCT, 3 pts developed acute GVHD (aGVHD) of grade I (n=1) or II (n=2), at a median of 26 days after haploHSCT. The median time from haploHSCT to first DLI was 204 days (range: 71–624). All pts had full donor chimerism at time of DLI. Before DLI 3 pts relapsed at a median time of 149 days (range:86–177), of whom 2 pts had AML and received salvage chemotherapy and 1 pt with HL being treated by DLI alone. Of the 3 relapsed pts, 1 showed progressive disease after first DLI dose and 2 achieved a sustained CR (with duration of CR of 6 and 9 months at last follow-up). The remaining 4 pts were given DLI in CR, in 1 case (of AML) associated with azacitidine. 5 pts received 1 DLI dose and 2 pts were given 3 DLI injections with escalating doses. The first dose of DLI was  $1 \times 10^6$  CD3/kg in 4 pts,  $5 \times 10^5$  in 1 pt and  $1 \times 10^5$  in 2 pts. The 2 pts who received 3 DLI doses (lymphomas) were given: (1)  $5 \times 10^5$ – $1 \times 10^6$ – $5 \times 10^6$ ; (2)  $1 \times 10^5$  for 2 doses followed by 1 dose of  $5 \times 10^5$ . Four pts developed chronic GVHD (cGVHD, 57%) in a median time of 23 days (range:11–42) after DLI (3 of them had presented previously aGVHD grade I–II). cGVHD was limited in 1 case, moderate in 1 pt and severe in 2 pts. 3 of these pts presented features of an overlap syndrome (acute/chronic GVHD) with signs of aGVHD de grade I,II and III in 1 pt each. Involved organs were skin/mucosal (n=4), liver (n=3), gastrointestinal tract (n=2), lung (n=1) and joints (n=1). All patients experiencing GVHD after DLI were treated by systemic corticotherapy, extracorporeal photopheresis and cyclosporine or weekly low dose methotrexate. Median follow-up after first DLI was 10 months (range:4–34). None of the 4 pts receiving prophylactic DLI relapsed during the follow-up period. 2 pts died, 1 of relapse and 1 of severe cGVHD. 5 pts were in CR at last follow-up, 3 with no signs of GVHD and 2 with limited cGVHD. Despite the limited cohort, DLI after HaploHSCT appears to be a therapeutic option in high risk pts allowing enhancement of GVT in the setting of haploHSCT with post-Cy infusion.

**Disclosure of conflict of interest:** None.

#### P074

Previously published

#### P075

**Early and sequential CTLA4Ig primed donor lymphocyte infusions (DLI) following post-transplantation cyclophosphamide (PTCY)-based haploidentical PBSC transplantation for advanced hematological malignancies promote proliferation of mature natural killer (NK) cells with cytotoxic potential and markedly reduces relapse-risk without increase in GVHD**

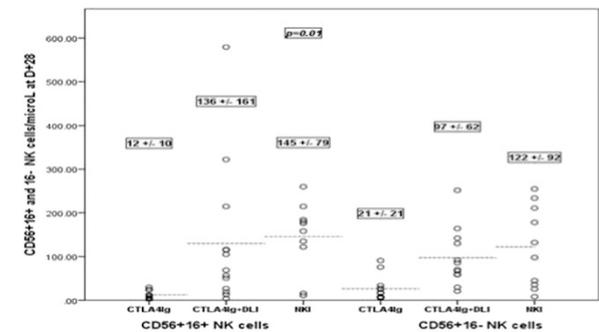
SR Jaiswal, S Zaman, P Bhakuni, S Bansal, S Deb, S Bhargava and S Chakrabarti

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We have earlier shown that CD56 enriched cell infusion following PTcy resulted in rapid proliferation of mature NK cells with attenuation of GVHD and early use of prophylactic G-CSF mobilized DLI resulted in improved disease-free survival. CTLA4Ig has been shown to be effective in attenuating T cell activation and induce transplantation tolerance in preclinical models. It has recently been employed to induce transplantation tolerance and reduce early alloreactivity in patients with nonmalignant disorders undergoing PTcy-based haploidentical HSCT. NK cells on the other hand are resistant to CTLA4Ig and in fact might demonstrate better anti-tumour effect in presence of CTLA4Ig as CD86 is a putative activation receptor. To explore this phenomenon, we employed sequential CTLA4Ig primed DLI following PTcy-based haploidentical HSCT in patients with relapsed/refractory hematological malignancies. 15 patients (12–60 years; AML-6, ALL-5, NHL-4)

received Abatacept (CTLA4Ig) as a part of GVHD prophylaxis at 10 mg/kg on day -1 followed by PBSC and sequentially on days +6, +20 and +35 followed 12 h later by DLI of  $5 \times 10^6$  CD3 cells/kg containing  $0.03$ – $1 \times 10^6$ /kg CD56+ cells. PTcy was administered on days +3 and +4 with cyclosporine from day +5 to day +60 and subsequent rapid tapering. The immune reconstitution of the study group (CTLA4Ig-DLI) was compared with the cohort of patients with both malignant and nonmalignant diseases who received Abatacept but not DLI (n=12; CTLA4Ig group) and those receiving CD56 enriched donor cell infusion on day +7 (n=10; NKI group). **Results:** There were no acute infusion related toxicities. All patients engrafted at a median of 15 days (12–20 days). The incidences of acute and chronic GVHD (all mild) were 20% and 25%, respectively. Three patients reactivated CMV and there was only one non-relapse mortality (6.9%). Only 4 patients relapsed (27.8%) with a disease-free survival of 72.6% at 1 year. These cells had greater expression of CD107a compared to normal healthy donors. The recovery of CD56+, CD56+16+ and CD56+16- cells were similar in the CTLA4Ig-DLI and NKI groups at days 28, 60 and 90 post-transplant and this was significantly higher than the CTLA4Ig group (Figure 1). In contrast to CTLA4Ig group, NK cells recovered at day +28 with predominantly CD56dim CD16+ phenotype with significant population of cells expressing KIR+NKG2A phenotype in both CTLA4Ig and NKI groups with higher expression of CD107a. Interestingly, the 4 patients who relapsed had attenuated recovery of CD56+16+ cells at 28 and 60 days (21/ $\mu$ L and 15 cells/ $\mu$ L) without CD107a expression, in contrast to the rapid and sustained recovery of this population of NK cells in those not experiencing relapse (CD56+16+ cells 181/ $\mu$ L and 103/ $\mu$ L). However, the recovery of Tregs was prompt and sustained in the comparator groups, which remained low in the CTLA4Ig-DLI group until day +90. There were no differences in the recovery of other T cell subsets between the three groups. The study demonstrates the unique ability of CTLA4Ig to augment NK cell proliferation, maturation and cytotoxicity and reduce relapse with attenuation of T cell activation and GVHD in the context of the early use of CTLA4Ig primed DLI following PTcy-based haploidentical HSCT without *ex vivo* selection or expansion. We hope this novel strategy might offer less expensive and yet a viable alternative in the field of NK cell therapy.

[P075]



**Disclosure of conflict of interest:** None.

#### P076

**Enhanced cytotoxicity of  $\gamma\delta$ -cytokine induced killer cells against hematologic malignancies**

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CIK cells are *ex vivo* expanded by scheduled addition of anti-CD3 mAbs and a cytokine cocktail that contains IFN- $\gamma$ , IL-2 or IL-15. Cells represent an *in vitro* generated heterogeneous population consisting of different effector cells—CD3posCD56pos, CD3negCD56pos and CD3posCD56neg-T cells that mainly (>95%) express  $\alpha/\beta$  T-cell receptor (TCR)

and to a lesser extent (< 5%), TCR  $\gamma\delta$  phenotype. These NK-like T cells product show a dual functional activity, retaining their original T cell specificity and NK cytotoxic capacity via marked up regulation of the NK cell receptor, NKG2D. Pre and clinical studies showed that the optimal cytotoxic effect of CIK cells against different malignancies (target cells) is achieved at 40:1 E:T ratio, which means high numbers of  $\alpha\beta$  T-cells that might increase the risk of GvHD. Here we produced CIKs from  $\alpha\beta$  TCR depleted cellular products (defined as  $\gamma\delta$ CIK) and tested their phenotype expression and *in vitro* cytotoxic activity against hematological malignancies. Fresh apheresis products were processed using the CliniMACS depletion reagent, according to manufacturer instructions. Target product was cultured with RPMI1640 supplemented with 10% FCS and *ex vivo* expanded by scheduled addition of cytokine cocktail that contains IFN- $\gamma$  (1000 IU/mL), anti-CD3 mAbs (50 ng/mL) and 500 IU/mL IL-2. The cells were cultured for 14 days. Cytotoxic activity of the  $\gamma\delta$ CIK was evaluated against various target hematological malignant cell lines (K562, REH, Jurkat, and U937). After 14 days, the  $\alpha\beta$  depleted CIK cultures resulted in 97.5%  $\gamma\delta$  T-cells (41 folds expansion) compared to 1.0% of  $\gamma\delta$  T-cells immediately after depletion, and compared to only 1.6% in non-selected CIK cells. The percentage of  $\alpha\beta$  T cells in  $\gamma\delta$ CIK cell cultures started from 0.002% (immediately after depletion) to 0.5% compared to 95.1%  $\alpha\beta$  T cells were found in non-selected CIK cells cultures.  $\gamma\delta$ CIK cells produced robust cytotoxic activity at a 10:1 E:T ratio against REH cells (22.6  $\pm$  5.3%), Jurkat cells (51  $\pm$  7.9%); U937 (62.5  $\pm$  8.5%) and K562 (43.4  $\pm$  2.0%), compared to non-manipulated CIK cell activity against the same targets (5  $\pm$  1.0%; 8.3  $\pm$  1.4%; 12.4  $\pm$  6.1%; 7.3  $\pm$  2.9%, respectively). We found higher degranulation capacity of  $\gamma\delta$ CIK cells compared to non-selected CIK cells against REH (45.3  $\pm$  16.1% vs 17.6  $\pm$  3.8%), Jurkat (42.3  $\pm$  18.4% vs 6.8  $\pm$  3.5%), U937 (37.6  $\pm$  15.5% vs 17.1  $\pm$  3.1%) and K562 (29.2  $\pm$  16.5 vs 10.3  $\pm$  2.9%) leukemic cells. Moreover,  $\gamma\delta$ CIK showed lower expression of exhaustion markers PD1, LAG3 and TIM3 compared to higher expression on non-selected CIK cells and  $\alpha\beta$ CIK cells.  $\gamma\delta$ CIK cells demonstrated an enhanced cytotoxic activity against a variety of hematological malignant cell line compared to non-selected CIK cells at a low E:T ratio (10:1). Moreover,  $\gamma\delta$ CIK cells present lower expression of exhaustion and inhibitory receptors, compared to non-selected CIK cells and  $\alpha\beta$ CIK cells. As significant reduction of  $\alpha\beta$  T-cells number reduces the risk of GVHD,  $\gamma\delta$ CIK cells product may offer a platform for safe and effective immunotherapy in the post-transplant setting.

**Disclosure of conflict of interest:** None.

#### P077

### Feasibility and safety of allogeneic *ex vivo* activated-NK cell infusion after matched related hematopoietic stem cell transplantation: Preliminary results of a prospective phase I trial

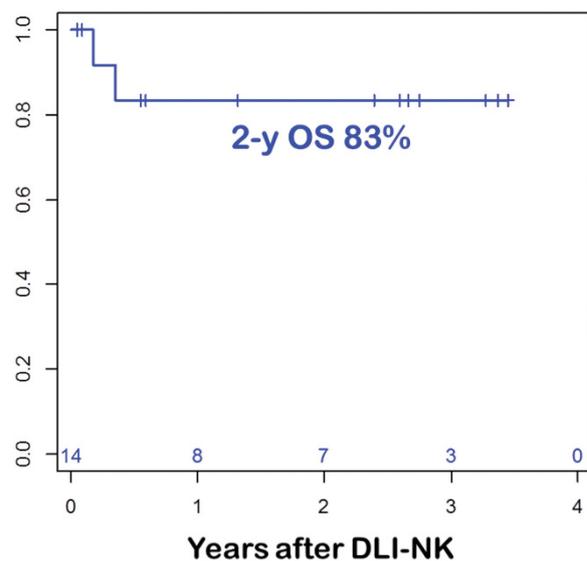
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During the past 15 years, the major improvements in the field of allogeneic hematopoietic stem cell transplantation (HSCT) (reduced intensity conditioning regimen, high level HLA typing, alternative donors, GvHD prophylaxis...) significantly extended the feasibility of this procedure. In contrast, disease recurrence after HSCT remains a main issue. Thus, many post-HSCT prophylactic interventions are under investigation. Unmanipulated donor lymphocyte infusion (DLI) remains one of the most frequently used post-HSCT treatment, but its potential benefit in increasing GVL effect may be

counterbalanced by the induction of GvHD. In this setting, the use of adoptive transfer of *ex vivo* enriched and activated NK cell infusions from the same donor (DLI-NK) may induce GVL effect without causing GvHD. We therefore report on a single-center Phase 1 clinical trial (NCT01853358) evaluating the safety of *ex vivo* activated allogeneic NK cells infused between days 60 and 90 after HSCT. The aim was to determine the maximum tolerated dose (MTD) of *ex vivo* highly purified and activated DLI-NK after matched related donor HSCT. The schedule plan a first phase of 3+3 dose escalation method using 3 dose levels (1.10e6/kg, 5.10e6/kg and > 5.10e6/kg). Grade 3-4 secondary adverse events according to NCTCI classification and severe GvHD occurring within 30 days after DLI-NK were considered as dose-limiting toxicities (DLT). A second step allowed enrolling patients at the MTD. Over a period of 3.5 years, 14 patients with various hematological malignancies (AML, ALL, HL, NHL, MDS) were infused with activated NK cells at a median time of 91 days (range: 61-106) post-HSCT. Apheresis products were collected from the HSC donor, CD3-depleted and CD56-selected by immunomagnetic separation using CliniMACS. Selected NK cells were cultured for 7 days in medium supplemented with 10% fetal calf serum in the presence of 1000 U/mL of IL-2 in air-permeable cell culture bags. After immunomagnetic separation, CD56-enriched products had a median CD56+ cell purity of 94% (range: 77-100) and viability of 96% (93-99). After IL-2 activation, the median CD56+ cell dose was 4.8  $\times$  10<sup>6</sup>/kg (1.2-21.4), with a viability of 81% (71-94) and a residual CD3<sup>+</sup> cell content of 0.4  $\times$  10<sup>4</sup>/kg (0-1.5  $\times$  10<sup>4</sup>/kg). All release criteria to be met were fulfilled for the 14 preparations infused: viability > 90%, negative microbiological testing, CD56<sup>+</sup> cell count  $\geq$  1  $\times$  10<sup>6</sup>/kg, and CD3<sup>+</sup> cell content < 5  $\times$  10<sup>4</sup>/kg. Standardized quality controls were employed at all steps of the manufacturing process, adding a level of consistency to the product testing before release. Activated-NK cells were well tolerated in all 14 patients, with no occurrence of DLT. Thus, MTD was not reached. Two patients presented with a moderate chronic GvHD, both of them during cyclosporine A dose reduction. Relapse occurred in 2 patients with AML. One patient died from idiopathic pneumoniae, without evidence of relapse, GvHD or infectious disease. With a median follow up of 30 months (1-41), 2 year OS was 83% (Figure 1). Therefore, infusion of highly purified, activated-NK cells of donor origin as a substitute to standard DLI does not induce GvHD nor other side effects after HSCT: the demonstration that modulation of NK cell activity can achieve disease control after HSCT deserves to be investigated in larger trials.

[P077]



**Disclosure of conflict of interest:** None.

**P078****Feasibility, safety, rapid production and efficacy of institution-produced CD19 CAR T cells in refractory B cell malignancies in Israel**

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CAR T cells targeting CD19 are evolving to become a powerful tool for relapsed or refractory B cell malignancies. It is undetermined whether the production process can be accomplished in academic centers, or whether a centralized industry-driven production is preferred. We share our results of production and clinical experience with the first clinical-grade manufactured CAR T cells in an academic tertiary medical center in Israel. Clinical grade CD19 CAR production was verified on 7 dry runs. Five patients were approved for treatment on compassionate use, all with refractory and relapsed B cell malignancies. Leukapheresis was performed locally and product was transferred to an on-site GMP facility. T cells were activated using OKT3 and IL-2 and transduced with a gammaretroviral vector encoding a second generation CD19 CAR based on the NCI vector (Kochenderfer *et al*, Blood 2010). Patients received a lymphodepleting conditioning regimen (fludarabine 25 mg/m<sup>2</sup>/day days -4 to -2, cyclophosphamide 900 mg/m<sup>2</sup> on day -2), followed by a target dose of 1 × 10<sup>6</sup> CAR+ T cells per kilogram on day 0, and admitted till resolution of neutropenia, fever and any symptoms related to cytokine release syndrome (CRS). As of December 2016, five children and young adults were treated for refractory relapse B-cell malignancies and had at least 30 days follow-up. The median age was 25 years (range: 7–43) and the median follow-up period was 67 days (range: 32–151). Two patients had prior ALL and 3 had DLBCL. The median number of prior therapeutic regimens patients received prior to CAR was 4 (range: 3–7) and three patients had a prior hematopoietic stem cell transplant. Production turnover time was an average of 9 days, including verification of *in vitro* activity in all products. Products were infused fresh from culture, and the target dose of CAR+ T cells was met in 4 of 5 patients (80%) with one patient receiving half the target dose. Four patients developed CRS grade 1–3, and only one experiencing grade 3 CRS requiring vasopressor support and administration of tocilizumab. Molecular detection of CAR+ T cells in the patients' peripheral blood was achieved in all patients, with peak CAR expansion seen on day +14. Significant toxicities in the first 30 days post CAR administration included CRS, febrile neutropenia (*n*=2), and 1 patient required transfusion for symptomatic anemia. No neurologic toxicities were documented. All four patients who had grade 1–3 CRS had a complete response, with negative MRD levels in the patients with ALL. One patient had no CRS, and on day +28 showed progression of his lymphoma despite receiving the target CAR T cell dose and initial *in vivo* CAR T-cell expansion. One patient received donor-derived CAR T cells from his HLA-matched brother without lymphodepletion. This patient had complete remission of his lymphoma, but went on to develop severe liver GVHD complicated by a viral infection resulting in death while in remission. All patients with a CR were referred to an allogeneic HSCT within 60 days of CAR therapy. We have demonstrated feasibility and efficacy of in-house clinical-grade CAR T cell production and administration in a tertiary medical center, with a rapid turnover time and an 80% remission rate. A clinical trial to further evaluate the safety and toxicity of our CD19 CAR production is ongoing (NCT02772198).

**Disclosure of conflict of interest:** None.

**P079****Fostering a public cord blood bank in Jordan: King Hussein cancer center experience and prospective for the future**

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Umbilical cord blood (UCB) is a common alternative source hematopoietic stem cell for allogeneic transplantation. Over than 700 000 UCB units have been stored and listed on the Bone Marrow Donor's Worldwide Registry, and more than 30 000 UCB transplants have been performed. Background: In pursuit of efforts to expand our alternative donor program, we share our initial experience in establishing the first public cord blood bank in Jordan, highlighting the efforts to solicit donations to build the infrastructure of the program, as well the analysis of the characteristics of collected and processed first 101 UCB units. Establishing and hosting a national public cord blood bank was put forth as an initiative of King Hussein Cancer Center (KHCC) in 2012. A cord blood bank temporary facility was built in 2015. Major equipment such as Bioarchive cryopreservation, AXP and Sepax automated cell separation devices were all obtained through donations. An agreement with a local private/charity hospital was made for UCB collection. Staff were trained on site for collection, processing and cryopreservation by regional experts. A total of 101 units were collected and processed as part of the initial validation of the project. UCB units were processed on either AXP or Sepax systems, and all cryopreserved in Bioarchive (an automated, robotic cryopreservation system that can archive up to 3623 units). The characteristics of which as well as the post processing data are depicted in Table 1.

[P079]

UCB Units Characteristics	Pre-Processing Data	Cell Separation using AXP	Cell Separation Using Sepax
#Units collected	101	-	-
#Units processed	101	50	27
WBC/mL (10E06)	7.8± 3.0	NA	NA
RBC mL/UCB	NA	33±9.8	37.5±11.7
% HCT	31.5± 6.3	NA	NA
TNC (10E06)	833± 465	852±471	975±505
%CD34+	0.31± 0.2	2.4±2.0	2.8±1.7
Total CD34+ (10E06)	2.6± 2.0	NA	NA
%Contamination	0	0	0
%Viability	98.5± 1.8	97±2.1	95.7±2.9
%Recovery (TNC)	NA	73.7±12.9	83±15.2
Recovery (CD34+)	NA	93.1±23.4	107±29.9
RBC in Final Product (ml)	NA	7.9±0.9	12.1±1.7

We shared a successful story of establishing the first public cord blood bank in Jordan. The first 101 units collected showed excellent sterility, viability, collection volume and total nucleated cells. A very good recovery of both nucleated and CD34+ cells were obtained using AXP and Sepax cell separation systems. The process of validation of equipments and methodology is complete. We anticipate moving to permeant facility of the cord blood bank in the new expansion in early 2017. We look forward for steady progress in UCB recruitments, HLA typing, cryopreservation and adherence to NetCord-FACT standards as well as participation in international registries.

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**Disclosure of conflict of interest:** None.

**P080****Functionally active IFN-gamma secreting CMV pp65 specific T cell therapy as an alternative for clinically urgent CMV related diseases**

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Cytomegalovirus (CMV) related diseases are a serious cause of morbidity and mortality following hematopoietic stem cell transplantation (HSCT). It has been reported over the last two decades that CMV-specific cytotoxic lymphocytes (CMV-CTLs) can provide long-term CMV-specific immunity without major side effects as an alternative to antiviral drugs. However, its application has been limited by prolonged manufacturing process of cell therapy. In this study, we apply the IFN- $\gamma$  cytokine capture system (CCS) using the fully automated CliniMACS Prodigy device to rapidly produce CMV-CTLs that may be applicable in clinically urgent CMV-related diseases. Five validation runs were performed using apheresis samples from randomly selected CMV-seropositive healthy blood donors. Then, CliniMACS Prodigy automatically performed successive processes including antigen stimulation, anti-IFN- $\gamma$  labelling, magnetic enrichment, and elution which took ~13 h. The original apheresis samples consisted of 0.3% IFN- $\gamma$  secreting CD3+ T cells in response to CMV pp65 antigen (CD3+IFN- $\gamma$ + cells) which were mainly CD45RA+CD62L+ naive T cells. Following IFN- $\gamma$  enrichment, the target fraction contained 51.3% CD3+IFN- $\gamma$ + cells with reduction in naive T cells and the selection of CD45RA-CD62L- and CD45RA+CD62L- memory T cells. Furthermore, extended culture of these isolated cells revealed functional activity including efficient proliferation, sustained antigen-specific IFN- $\gamma$  secretion and cytotoxicity effect against pp65 pulsed target cells. Therefore, we suggest IFN- $\gamma$  CCS by CliniMACS Prodigy as a simple and robust approach to produce CMV-CTLs, which may be highly feasible and applicable in clinically urgent CMV-related diseases.

**Disclosure of conflict of interest:** None.

**P081****In vitro generation of tumor antigen-specific T cells from patient and healthy donor stem cells**

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Acute myeloid leukemia remains a therapeutical challenge, as many patients relapse after chemotherapy. Allogeneic stem cell transplantation is in most of these patients the only option for cure, but carries a high risk of morbidity and mortality and a suitable donor may be lacking. Recently, advances are being made in the field of T cell immunotherapy. The classical protocol, in which peripheral blood lymphocytes (PBL) are transduced with a tumor antigen-specific T cell receptor (TCR), can generate T cells with low and possibly hazardous specificities (due to mispairing of the endogenous and introduced TCR  $\alpha$  and  $\beta$  chains). Therefore, we have developed a novel protocol in which we generate tumor antigen-specific T cells from CD34+ hematopoietic stem cells. We have already succeeded in generating large numbers of tumor-specific, naive and resting T cells that only carry the introduced TCR, starting from postnatal thymus and cord blood CD34+ cells. Now we are optimizing this protocol for clinically more relevant samples, such as mobilized peripheral blood from healthy stem cell donors and from patients in remission after chemotherapy and/or other treatments, and leukapheresis samples from patients at diagnosis. In our protocol, CD34+ cells were isolated from HLA-A2+ fresh patient and healthy donor samples and cultured on OP9-DL1 in the presence of SCF, FLT3L and IL-7, until T cell commitment. Subsequently, the cells were transduced with a tumor antigen-specific TCR and again co-cultured until CD4+

CD8+ double positive cells were abundantly present. At that point, agonist peptide was added, which induces maturation. Finally, cells were polyclonally expanded on feeder cells. For HLA-A2 negative samples, CD4+ CD8+ double positive cells were co-cultured with a cell line (T2 pulsed with the agonist peptide or a cell line with endogenous expression of the agonist peptide) which can present the agonist peptide to the maturing T cells. Using the above protocol, we were able to generate tumor antigen-specific T cells from 3 out of 3 healthy donor samples, 1/1 sample from a patient in remission and 2/4 samples from patients at diagnosis, who were all HLA-A2+. For most samples, multiple rounds of agonist peptide stimulation were necessary to obtain further maturation. In contrast, generation of mature T cells from CD4+ CD8+ double positive cells in postnatal thymus or cord blood co-cultures, requires only 1 round of agonist peptide stimulation. For the HLA-A2 negative samples, we were able to generate an adequate CD4+ CD8+ double positive population from 1/1 healthy donor sample, 3/3 samples from patients in remission and 0/1 sample from a patient at diagnosis. Agonist selection using a cell line seems inefficient as CD27 is not upregulated and cells did not mature to CD4+ or CD8+ single positive mature T cells. We are currently co-culturing more samples using our protocol. Furthermore, we are investigating the effect of freezing and thawing on the *in vitro* T cell generation process (cell numbers and efficiency). Finally, we are also working on optimizing the protocol for generation of tumor antigen-specific T cells from HLA-A2 negative patient and healthy donor samples.

**Disclosure of conflict of interest:** None.

**P082****Increase of polyspecific immune responses against leukemia-associated-antigens (LAA) and reduction of regulatory T cells frequency after donor lymphocyte infusion (DLI) in patients with hematological malignancies**

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Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-SCT) and/or donor lymphocyte infusions (DLI). Graft versus leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemia associated antigens (LAA) in different hematological malignancies. However, T cell responses after allo-SCT and DLI are not well characterized. In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific CD8+ T cells using ELISpot analysis and tetramer assays in 12 patients (5 patients (pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, NPM1mut, RHAMM, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients. In ELISPOT and tetramer assays, an increase in frequency and diversity of LAA-specific T cells was observed in all patients. Importantly, there was a significant increase from a median of 1 to 4 LAA-derived T cell epitopes ( $P=0.03$ ) in clinical responders (R) when compared to non-responders (NR). These positive results in R vs NR where

confirmed by tetramer-based flow cytometry assays, where an increase in frequency from 0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed. Interestingly, the frequency of Tregs in clinical responders decreased significantly from a median 72.9 % to 54.6 % ( $P=0.008$ ) while the frequency of Tregs kept stable over time in non-responding patients. T cell subset analysis did not reveal significant differences before vs after DLI administration. In cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI we found a shift towards proinflammatory and T cell stimulating cytokines. Taken together, we detected an increase of specific CTL responses against several LAA after allo-SCT and DLI. Moreover, this study suggests that enhanced LAA epitope-specific T cell responses as well as decreasing numbers of Tregs contribute to clinical outcome of patients treated with DLI.

**Disclosure of conflict of interest:** None.

#### P083

##### Previously published

#### P084

##### **Intra bone route of donor lymphocyte infusions in patients relapsing post alloHSCT may result with anti-leukemic effect associated with accumulation of CD279+ lymphocytes and the cells with a distinct TCRbeta clonotype in the marrow**

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Immunologic surveillance of leukemia is employed for the prevention and treatment of relapse post alloHSCT. To augment this effect donor lymphocytes are infused (DLI) in patients at risk. This procedure is associated with a high risk of aGvHD and we believe that this route of administration may not make the direct contact between infused cells and blasts the optimal one. To address these issues, we started delivering donor lymphocytes directly to the bone marrow cavity (IB-DLI) in patients post alloHSCT at relapse. Three with AML and one with CLL, all relapsed post alloHSCT: 3 alloSIB: 50-year-old female AML patient (relapsed 2 years post HSCT), 22-year-old AML male 7q31 del (relapsed in 3 years, traumatic brain injury), 25-year-old male AML FLT3 ITD+ received MUD HSCT (relapsed 9 months) and 64-year-old CLL male, TP53 del, EBV reactivation (progressed 7 years). Two patients (26% and 12% blasts in the marrow) received IB-DLI up-front and two others due to higher proportions of leukemic cells received either FLAG (AML case) or anti-CD20 MoAb (CLL case) followed by IB-DLI. Three to five IB-DLIs were performed starting from 10E6 and ending with a dose of 10E8 of CD3+ cells/kg body weight. Donor lymphocytes were obtained from leukopoietic product either unstimulated or the stimulating ones (J Hasskarl *et al.* 2012) used for transplantation. The cells were injected directly to the bone marrow cavity. The blood and marrow specimens were taken prior each IB-DLI for: cytology, cytometry (including CD8, CD279, CD26, CD28 MoAb in addition to the routine staining used for blast cells), genetic work (chimerism, mutations associated with the disease). No side effects were noticed, including GvHD symptoms. Anti-leukemic effect: all patients responded to the IB-DLI either with CR or PR. The response lasted from 11 to 22 months. In two cases the second transplant was performed with a fatal outcome 14 and 18 months after the first relapse when IB-DLI was initiated. The laboratory work-up: Collectively CD279+ cells contributed to the lymphocyte pool in the marrow to a greater extent than it was seen in the blood at the same time ( $16.6 \pm 2.9\%$  vs  $33.1 \pm 4.0\%$ ,  $P < 0.001$ ); this was also valid for CD8+CD279+ cells ( $12.4 \pm 3.2\%$  vs  $27.1 \pm 4.5\%$ ,  $P < 0.001$ ). TCR clonotyping revealed in all 4 patients the presence of the prevailing oligoclonal response on the polyclonal background

(characteristic for each individual) which was identified in the marrow and in the blood. However, in two out of 4 patients a distinct oligoclonal peak was seen at first in the marrow and then in the blood. Microarray analysis of the transcriptome in the marrows of patients who received three IB-DLI courses revealed in all patients preferential use of genes associated with lymphocyte or lymphocyte activation pathways. The patients who responded favorably (CR or PR) clustered with the transcriptomes of normal individuals, but those who failed to respond clustered separately. IB-DLI was safe and not associated with GvHD. Selective accumulation of CD8+CD279+ as well as the presence of a distinct oligoclonal peak in the marrow suggest that TCRbeta clonotypes may be private to leukemia cells recognition. The response may result in CR or PR and the patients were in a good physical shape during the treatment, which makes it possible to deliver the salvage chemotherapy if required.

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#### P085

##### **Intra-lesioner autologous stem cell application in diabetic foot/ulcer**

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The diabetic foot/ulcer which can result with amputation is one of most important complications of diabetes mellitus. Experimental and clinical studies have shown that bone marrow-mobilized stem cells have positive effects on diabetic foot/ulcer treatment. A 69-year-old diabetic male patient, admitted to hospital because swelling and bruising on wrist, necrosis on the fourth fingertip, and 3×4 cm necrotic area at the plantar surface in the middle of the third, fourth, fifth metatarsal line at left leg (Picture A). The lesion of under the foot was debrided. Broad spectrum antibiotics were started. After the orthopedic consultation, the fourth finger was amputated and amputation from the left ankle was recommended. A stem cell transplantation option was offered to patients and their relatives as one of the therapeutic approaches. Upon acceptance by patient, 10 µgr/kg of colony stimulating agent was started to patient. When the stem cell was 20/µL, the stem cells were collected. The obtained stem cell product was injected intra-lesionally (Picture B). Granulation tissue began to develop from the second week in the foot floor of the patient. After from 8th week, the necrotic tissue was disappeared and the granulation tissue was appeared. At 24 weeks, 50% of the lesion healed. At 48th week, there was normal tissue instead of necrotic tissue on plantar surface at left leg (Picture C). This case report suggests that diabetic foot/ulcer can be healed with intralesional application of stem cells in patients with diabetes mellitus.

[P085]



**Disclosure of conflict of interest:** None.

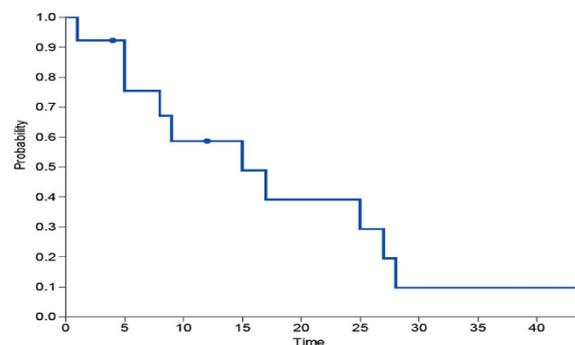
**P086****Micrografting HLA-partially matched mobilized cellular therapy (microtransplantation) in elderly patients with acute myeloid leukemia and myelodysplastic syndromes**

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Micro-engrafting HLA-partially matched mobilised peripheral-blood hematopoietic cellular therapy (microHCT) following conventional chemotherapy has been pioneered by Ai *et al.* as a treatment for elderly patients with acute myeloid leukaemia and myelodysplastic syndromes (AML/MDS). A host-vs-tumour effect induced by alloreactive graft-rejection is the proposed underlying mechanism. Here, we present an independent validation of this technology, with a particular focus on its feasibility following hypomethylating agents, which are normally used in such elderly AML/MDS patients. We have performed 34 microHCT infusions in a total of 15 microHCT procedures (5×3 infusions, 9×2 infusions and 1×1 infusion) in 12 AML/MDS patients who were not candidates for allogeneic HCT: median age a first microHCT 68.5 years (64–75); 6 AML, 2 CMML, 1 RAEB, including 3 secondary AML and 4 refractory/relapsed; from 15 partially mismatched donors (13 haploidentical). G-CSF-mobilized cell products included median numbers of  $2.98 \times 10^8$ /kg MNC (range: 1.37–6.59),  $3.20 \times 10^6$ /kg CD34+ (range: 1.05–10.72),  $1.06 \times 10^8$ /kg CD3+ (range: 0.46–2.42×10<sup>8</sup>/kg) and  $0.13 \times 10^8$ /kg NK (range: 0.02–0.19) cells infused per infusion, and  $6.96 \times 10^8$ /kg MNC (range: 4.52–10.70),  $7.31 \times 10^6$ /kg CD34+ (range: 3.2–15.6),  $2.59 \times 10^8$ /kg CD3+ (range: 0.64–3.53×10<sup>8</sup>/kg) and  $0.24 \times 10^8$ /kg NK (range: 0.15–0.33) cells infused per full microHCT procedure. Three patients had two microHCT procedures from different donors. First cell infusions (*n*=15) were fresh and all second (*n*=14) and third (*n*=5) cell infusions were cryopreserved. Cells were infused following conventional chemotherapy (IA, MEC, HDaC) in 15 cases (44%), chemotherapy plus hypomethylating agents in 8 cases (24%) and hypomethylating agents alone in 11 cases (8 azacytidine, 3 decytabine; 32%). The procedure was well tolerated, with mild and transient 'haploimmunostorm syndrome' (fever 84%, rash 28%, diarrhea 14%). Only the two patients with CMML received corticosteroid. One patient suffered early infusional reaction that was resolved with support treatment. None of the patients showed acute or chronic GVHD or persistent donor engraftment in chimerism tests. Four patients had bacterial infections, but no other significant invasive fungal or viral infections were observed. All AML/RAEB patients treated achieved complete remission with microHCT treatment (13; 87%). Only one patient, with CMML, died during microHCT induction (7%). Four patients relapsed at 7, 9, 10 and 15 months after the infusion; two of them achieved a second sustained complete remission with another micro-HCT from a different donor (one of them had developed anti-HLA antibodies). As described in Figure 1, median overall survival is 16 months and overall survival at 2 years is 40%. MicroHCT is a well tolerated procedure in elderly AML/MDS patients who are not candidates to allogeneic HCT. Infectious complications are insignificant and the remission rates are very encouraging in very high risk cases, with no evidence of GVHD. Patients can undergo a second microHCT from a different donor. In addition to the experience by Ai *et al.*, we have also shown that microHCT can be safely administered following a hypomethylant agent course instead of conventional chemotherapy. A large, international, randomized clinical trial will address the safety and efficacy of microHCT for elderly AML/MDS patients (NCT02171117).

**[P086]**

Figure 1. Overall Survival



**Disclosure of conflict of interest:** None.

**P087****Modulation of heme oxygenase-1 activity to enhance WT1-specific T-cell responses for immunotherapeutic approaches**

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Wilms Tumor protein 1 (WT1) is expressed in a variety of solid tumors and is found in more than 80% of patients with acute myeloid leukemia which makes it an attractive target for immunotherapy. Previously it was shown that T cells recognizing WT1 are suitable for adoptive T-cell therapy by increasing the graft versus leukemia effect. However, the efficiency of this therapeutic strategy is still limited due to the low precursor frequency and specificity of WT1-specific T cells in the peripheral blood of healthy donors. The ubiquitous antioxidant inducible enzyme heme oxygenase-1 (HO-1) and its products have immunomodulatory effects, which render it as a potential target for the modification of T-cell responses. Recently, we found that inhibition of HO-1 enzyme activity via tin-mesoporphyrin (SnMP) results in activation and proliferation of antiviral T cells from healthy donors. In this study we aimed (1) to identify the mechanism of HO-1 modification in the generation of WT1-specific T cells and (2) to develop strategies for the sufficient generation of WT1-specific T cells from healthy donors to augment effective T-cell immunity in leukemia patients and to broaden the applicability of adoptive T-cell therapy to the majority of patients. The frequency of WT1-specific T cells in peripheral blood of healthy donors (*n*=50) was examined before and after SnMP treatment via IFN-γ ELISpot using the WT1-overlapping peptide pool (ppWT1). Enrichment efficiency of WT1-specific T cells after HO-1 inhibition was verified in response to ppWT1 and the HLA-A\*02:01-restricted WT1 peptides 37 (VLDFAPPGA, WT137) and 126 (RMFPNAPYL, WT1126) by IFN-γ secretion assay and expression analysis of the T-cell activation marker CD137. Phenotypic and functional characterization of WT1-specific T cells were further assessed by multicolor flow cytometry, luminex assays and ELISA with respect to T-cell subsets, cytotoxicity, proliferative capacity and secretion of effector molecules. In 24% of donors we found specific T cells against ppWT1 by IFN-γ ELISpot (10 spots/250.000 PBMCs). The frequency of WT1-specific T cells in these donors could be increased fivefold after inhibition of the enzymatic activity of HO-1 via SnMP. To assess the possibility that HO-1 modulation might be clinically applicable in conformity with good manufacturing practice, enrichment of SnMP-treated WT1-

specific T cells was evaluated based on IFN- $\gamma$  secretion and CD137 expression. Compared to SnMP-untreated cells there was a 3.74-fold higher response of HO-1 modified WT1-specific T cells pre-enrichment and an up to 16-fold higher enrichment efficacy, while SnMP treatment did not affect the T-cell functionality. In conclusion, modification of the enzymatic activity of HO-1 resulted in a more effective generation of functionally active WT1-specific T cells suitable for adoptive T-cell therapy. This makes HO-1 a promising therapeutic target to boost antigen-specific T-cell responses for treatment of WT1-positive tumors.

**Disclosure of conflict of interest:** None.

#### P088

##### **Pathogen reduction through additive-free short-wave UV light irradiation retains the optimal efficacy of human platelet lysate for the expansion of human bone marrow mesenchymal stem cells**

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We recently developed and characterized a standardized and clinical grade human Platelet Lysate (hPL) that constitutes an advantageous substitute for fetal bovine serum (FBS) for human mesenchymal stem cell (hMSC) expansion required in cell therapy procedures, avoiding xenogenic risks (virological and immunological) and ethical issue. Because of the progressive use of pathogen reduced (PR) labile blood components, we evaluated the impact of the novel procedure THERAFLEX UV-Platelets for pathogen reduction on hPL quality (growth factors content) and efficacy (as a medium supplement for hMSC expansion). This technology is based on short-wave ultraviolet light (UV-C) and has the main advantage not to need the addition of any photosensitizing additive compounds (that might secondary interfere with hMSCs). We applied THERAFLEX UV-Platelets procedure on fresh platelet concentrates (PCs) suspended in platelet additive solution and prepared hPL from these treated PCs. We compared the quality of PR-hPL with the corresponding non-PR ones, in terms of growth factor contents. Then, we evaluated the efficacy of PR-hPL, in comparison with hPL and MSC-screened FBS. We performed large scale culture of hMSCs during 3 passages and evaluated the proliferation of cells and the maintenance of their properties: profile of membrane marker expression, clonogenic potential, immunosuppressive properties (inhibition of T-cell proliferation) and potential to differentiate in adipocytes and osteoblasts. We showed no impact on the content in 5 growth factors tested (EGF, bFGF, PDGF-AB, VEGF and IGF) and a significant decrease in TGF- $\beta$ 1 (-21%,  $n=16$ ,  $P < 0.01$ ). A large scale culture of hMSCs during 3 passages showed that hPL or PR-hPL at 8% triggered comparable hMSC proliferation than FBS at 10% plus bFGF ( $n=3$ ). Moreover, after proliferation of hMSCs in hPL or PR-hPL containing medium, their profile of membrane marker expression, their clonogenic potential and immunosuppressive properties (inhibition of T-cell proliferation) were maintained, in comparison with hMSCs cultured in FBS conditions. The potential to differentiate in adipogenic lineage of hMSCs cultured in parallel in the 3 conditions, evaluated using Oil Red O and Nile Red stainings and the measurement of triglyceride accumulation, remained quantitatively identical. We also showed that the potential to differentiate in osteoblasts (quantified using Alizarin Red S and Von Kossa stainings and

ALP activity measurement) of hMSCs grown in hPL or PR-hPL was not impaired, in comparison with FBS. In conclusion, we demonstrated the feasibility to use UV-C treatment to subsequently obtain pathogen reduced hPL, while preserving its optimal quality and efficacy for hMSC expansion for cell therapy applications.

**Disclosure of conflict of interest:** SV, LC, SE, FG, CS and BD are employees of Macopharma.

#### P089

##### **PEG-ADA induced thrombocytopenia in a SCID patient mandating hematopoietic stem cell transplant at late adolescence: A case report and review of literature**

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Adenosine deaminase (ADA) deficiency is one variant of severe combined immunodeficiency (SCID) which is an autosomal recessive primary immunodeficiency disease. In most cases, it leads to a severe combined immunodeficiency (ADA-SCID) manifested by dysfunctional T, B, and NK cells (T-B-NK-SCID) and showed early in life. ADA-SCID is managed in several ways including enzyme replacement therapy (ERT) with polyethylene glycol-modified adenosine deaminase (PEG-ADA) which is used for restoration of immune function with low rate of complications. Other modalities include hematopoietic stem cell transplantation (HSCT) from matched sibling donor (MSD) or matched family donor (MFD) that is considered a definitive treatment of choice for ADA-SCID. It has good outcomes when done early in life providing that infection and advanced immune compromise can be prevented. In contrast, matched unrelated donor (MUDD) has much less rewarding results particularly if done at later age with pre-existing complications. Recent modality for treatment is gene therapy (GT) although it is still not used widely in clinical practice. In this paper, we demonstrated a case of ADA-SCID who received HSCT as an adolescent from matched unrelated donor (MUDD) after termination of her PEG-ADA treatment due to severe intractable thrombocytopenia induced by PEG-ADA. Patient showed good engraftment and incremental clinical improvement. Her post transplantation course was complicated with multiple complications including: grade I gut GVHD as well as hemorrhagic cystitis (BTK related) and EBV infection. Additionally, she developed several CNS complaints like headache, vomiting and dizziness which were found to be due to increased intracranial pressure with multiple enhancing cerebral lesions found on brain imaging. Further investigations for the brain lesions confirmed the diagnosis of malignant diffuse large B cell lymphoma (DLBCL) involving the brain. The lymphoma was highly suggested to be originated from donor cells giving the timing relationship between transplant and establishment of the diagnosis. This Lymphoma was successfully treated with full recovery and good final immune reconstitution but with lack of B cell engraftment and need for monthly IVIG. We conclude that, PEG-ADA can rarely induce thrombocytopenia in an autoimmune manner by forming antibodies against platelets and good recovery of thrombocytopenia can be achieved after discontinuation of PEG-ADA. HSCT can be considered as modality of treatment even in older patients with SCID due to ADA deficiency keeping in mind high possibility of complications including, autoimmunity and malignancy.

**Disclosure of conflict of interest:** None.

**P090****PBSC mobilization and apheresis collection for autologous transplantation: Preliminary experience at the Hiwa cancer hospital, Sulaymaniya, Iraqi Kurdistan**

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The Hiwa Cancer Hospital (HCH) in Sulaymaniya was recently identified as the site for the establishment of the first HSCT center in the Iraqi Kurdistan. A project of the IUC funded by the Italian Cooperation Development Agency was started in April 2016, and soon a dedicated team started the PBSC mobilization and collection activity for autologous transplantation. Here we describe the initial experience and the results so far obtained. The apheresis devices were the Amicus (Fenwal) and the Comtec (Fresenius) cell separators. After collection and CD34+ cell enumeration, the samples were cryopreserved in 10% DMSO and stored in a -80°C freezer or in liquid nitrogen. Thawing was done in a 37°C water bath, and cells were infused following high-dose therapy without any prior manipulation. We report the results in 17 patients, 11 M and 6 F, median age 36 years (28–58), with multiple myeloma (MM, N=6), Hodgkin lymphoma (HL, N=9) and non-Hodgkin lymphoma (NHL, N=2). As mobilization regimen, all MM patients received G-CSF alone at the dose of 10 µg/kg/day for 5 days. The HL and NHL patients, who were already on salvage treatment, were collected after the same chemotherapy followed by G-CSF 10 µg/kg/day. In HL patients the chemotherapy was BeGeV (Santoro A. *et al.*, 2016) in 6, cyclophosphamide + G-CSF in 2, and IGeV in 1. The two NHL patients received cyclophosphamide + G-CSF or R-DHAP + G-CSF. PBSC collections were started at the time of rapid WBC rise and as soon as CD34+ cell count increased over baseline (median 30/ mL (8–236)). The apheresis collections were run through a peripheral vein or a femoral catheter by processing up to 2.5–3 times patient's total blood volume. To plan the apheresis procedures a published algorithm (Pierelli L. *et al.*, 2006) was regularly employed. Since in this first series of patients a single autograft was planned, the target for PBSC collection was set at  $5 \times 10^6$ /kg CD34+ cells for all patients. With this technology, the collection target was reached in 15 out of 17 patients (88%). In those who reached the target, we collected as median  $6.08 \times 10^6$ /kg CD34+ cells (2.4–20.8), with median 2 apheretic runs (1–4). Based on the pre and post-apheresis CD34+ cell counts, the collection efficiency of the apheresis Amicus device was median 89.5% (54–170) and of the Comtec median 82% (32–95). In MM the apheretic collections were started on median day 5 (4–6), while in lymphoma patients, due to chemotherapy, the day of apheresis start was 12 (9–18). After cryopreservation and thawing, viability (7-AAD, BD) was median 87.5% (43–100). With these cell products, up to now we engrafted 9 patients following high-dose chemotherapy (5 MM autografted after MEL200, 2 HL and 2 NHL autografted after BEAM). Engraftment was prompt and stable in all with ANC 0.5 and  $1.0 \times 10^9$ /L on median day 11 (10–12) and 12.5 (11–15), respectively, and with platelet count 20 and  $50 \times 10^9$ /L on median day 14 (11–17) and 17.5 (13–44), respectively. These results are similar to those obtained by most experienced centers in Europe and US, and confirm the fact that autologous transplantation may be implemented also in developing countries when appropriate technology and application of standard procedures are employed. With this experience our center is also developing allogeneic transplantation, and the initial results in thalassemia will be reported in a separate abstract.

**Disclosure of conflict of interest:** None.

**P091****PiggyBac transposon, a non-viral gene transfer system, mediated T cells expressing CD19 chimeric antigen receptor for a 'first in human' clinical trial**

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Chimeric antigen receptor (CAR)-modified T cells targeting CD19 have exhibited marked activity in hematological malignancies, such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia and B-cell lymphomas, and complete remission rate in ALL is reported as 70–90 %. Many of these results have been obtained using CD19-CAR T cells produced by retro- or lentiviral vectors, which have a potential risk of integration into or near proto-oncogenes. On the other hand, piggyBac transposon system have been shown to transduce a gene of interest into the genome randomly by cut-paste mechanism of the transposase. Using this system, we developed a non-viral vector-mediated CD19-CAR T cells, and confirmed their cytotoxic activity on several cancer cell lines *in vitro* and *in vivo*. Here we established a manufacturing method via piggyBac transposon system to provide clinical grade CD19. CAR T cells. CD19.CAR-T cells were produced from peripheral blood mononuclear cells (PBMC) of healthy donors by nucleofection using two vectors encoding CD19.CAR and piggyBac transposase, respectively. We used a 4D-Nucleofector device to electroporate  $1 \times 10^7$  peripheral blood mononuclear cells with a CD19.CD28.ζ-CAR transposon plasmid, and a piggyBac transposase plasmid. All cells were cultured in serum-free medium (TexMACS) containing IL-7 and IL-15 in 24-well plates. Electroporated cells were immediately transferred to irradiated autologous activated T-cells (ATCs), pulsed with 4 viral peptide pools (PepTivator; AdV5 Hexon, CMV pp65, EBV EBNA-1, and BZLF1) (ACE). The next day, cells were transferred to CD3 and CD28 antibody-coated plates for 5 days. On day 7, all cells were transferred into G-Rex10 culture flasks with ACE-pulsed irradiated ATCs. On day 14, we collected CAR-T cells from all conditions. We conducted pre-clinical experiments *in vitro* and *in vivo* mouse model for a clinical trial. We detected the integration sites of CAR genes by inverse polymerase chain reaction and subsequent next-generation sequencing using MiSeq. An analytic pipeline was developed to identify and to classify the integration sites. Proto-oncogenes were defined according to the Cancer Gene Census of the Catalogue of Somatic Mutations in Cancer database. The median number and transduction efficiency of CAR-T cells obtained from  $2 \times 10^7$  PBMC in 9 donors were  $1.0 \times 10^8$  (range:  $0.58-1.8 \times 10^8$ ) and 51% (range: 29–73%), respectively. The major subset of CAR-T cells was phenotypically CD8+CD45RA+CCR7+, closely related T-memory stem cells. *In vitro* CD19.CAR-T cells exerted potent cytotoxic effect on CD19 positive tumor cell lines. In NSG mice model, CD19. CAR-T cells dramatically inhibit tumor growth (Figure 1). CAR gene integration sites were determined by inverse polymerase chain reaction and subsequent next-generation sequencing using MiSeq and equally distributed throughout the genome without preference for specific sites. The pre-clinical testing in mouse demonstrated safe toxicity profile even at the 166 times dose of CD19.CAR-T cells supposed to be given for our clinical trial. The transduction efficiency of the CAR transgene in our modified piggyBac transposon, a non-viral gene transfer system, was as high as that in conventional virus vector system. These preclinical data support a piggyBac transposon technology as cost-effective and safe therapeutic platform for CD19.CAR-T cell therapy for the first-in-human clinical trial.

[P091]

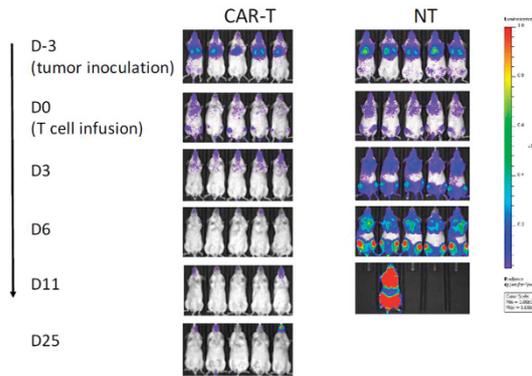


Figure. *PiggyBac* transposon mediated CD19.CAR T-cells against CD19 expressed leukemic cells :CAR-T vs Non-modified T (NT) cells

**Disclosure of conflict of interest:** None.

**P092**

**Relapse after allo-SCT is associated with high PD-1 and TIGIT expression on tumor-reactive CD8+ T cells**

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Allogeneic stem cell transplantation (allo-SCT) can be a curative treatment for patients with a hematological malignancy due to allo-reactive T cell responses recognizing minor histocompatibility antigens (MiHA) expressed by the patient's malignant cells. Despite the long-term presence of MiHA-specific memory CD8+ T cells, many patients eventually relapse. The failure to launch productive graft-versus-tumor T cell immunity can be attributed to tumor immune escape mechanisms, including dysregulated immune checkpoint signaling. Here, tumor cells impair T cell functionality by down-regulating expression levels of co-stimulatory (CSM) and up-regulating co-inhibitory molecules (CIM). The sum of these co-signals eventually determines the T cell activation state; a disbalance can contribute to tumor immune escape. The aim of this study was to evaluate co-expression profiles of immune checkpoint molecules on tumor-reactive T cells in respect to clinical outcome post allo-SCT. A 13-color flow cytometry panel was established to analyze co-expression levels of various CIM and CSM on CD8+ and CD4+ T cell memory subsets. Furthermore, we analyzed the expression levels on virus- and MiHA-reactive CD8+ T cells of healthy donors and allo-SCT patients. MiHA-reactive CD8+ T cells exhibited an early memory phenotype: high CD27 and CD28, and low KLRG-1 and CD57 expression. Interestingly, these T cells also displayed high co-expression of CIM such as PD-1, TIM-3 and TIGIT as compared to the Tem or virus-specific CD8+ T cells of allo-SCT patients. Most importantly, high expression of PD-1, TIGIT, OX-40 and KLRG-1 on MiHA-reactive CD8+ T cells was associated with relapse after alloSCT. Together, these data demonstrate that MiHA-specific CD8+ T cells of patients who relapse have a distinct CIM expression profile as compared to patients who stay in remission. These findings allow additional immunomonitoring after allo-SCT, and provide rationale for personalized adjuvant therapy, interfering with immune checkpoint signaling, to boost graft-versus-tumor immunity in patients with hematological malignancies.

**Disclosure of conflict of interest:** None.

**P093**

**Standardization method to characterize the key parameters influencing the extracorporeal photopheresis efficacy: Towards a better understanding of the procedure**

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Extracorporeal photopheresis (ECP) is a cellular immunotherapy treatment based on autologous infusion of apoptotic peripheral blood mononuclear cells (PBMC). ECP involves the exposure of PBMC to the photosensitizing agent (8-methoxypsoralen or 8-MOP) and UVA irradiation. Upon UVA irradiation, 8-MOP binds covalently to the DNA, subsequently stops the proliferation, and induces apoptosis of cells, mainly T-cells [1]. Even if ECP is largely used in clinic for a number of T-cell-mediated diseases, little is known about the influence of the different components inside the treated cell preparation. Here we studied the relative importance of those components: 8-MOP concentration, hematocrit levels, cell amount, plasma concentration and, the irradiation dose parameter. We used the human JURKAT T-cell line to standardize the evaluation. The technique efficacy was addressed using 2 different criteria reported in the literature: 70% proliferation inhibition 3 days after irradiation [2] and 15% apoptosis (%Apoptosis<sub>Treated</sub>-% Apoptosis<sub>Control</sub>) after 1 day [3]. JURKAT cells (Sigma Aldrich) were kept in culture in RPMI 1640 medium supplemented with 10% FBS, L-Glutamine and penicillin/streptomycin. Experiments were conducted using Theraflex ECP materials (MacoPharma): MacoGenic G2 UVA irradiation device, UVA irradiation bag, 8-MOP, ACD-A, and saline solution. Plasma and red blood cell concentrate were obtained from the French blood establishment (EFS). Cell death was assessed every day for 3 days after the irradiation by Annexin-V-FITC and Propidium iodide (BD pharmlingen) double staining using a flow cytometer (BD). Cell proliferation was determined by cell counting on day 3, 4 and 7 after irradiation using an automated cell viability analyzer (Beckmann Coulter). 8-MOP concentration before and after irradiation was measured using an HPLC (Shimadzu). The One-way ANOVA test was used:  $P < 0.05$  was considered as significant. Hematocrit (HCT) percentage was identified as the main critical factor influencing *in vitro* ECP efficacy. HCT levels up to 2% gave rise to an efficient ECP treatment at 2 J/cm<sup>2</sup>. An increase in irradiation dose counteracted the increase level of HCT. The level of 4% HCT imposes the use of 4 J/cm<sup>2</sup> to reach our two defined validation criteria. Dosage of 8-MOP demonstrated that differences of concentration before and after irradiation (~10% loss) remained stable in the tested conditions (% hematocrit and 8-MOP concentration) at 2J/cm<sup>2</sup>. Both concentrations of 8-MOP assessed (200ng/mL or 333ng/mL) were validated according to our acceptance criteria. In this study, a human T-cell line enabled us to get standardized results in order to highlight the key parameters that influence the biological efficiency of the technique. Moreover, we found ways to counteract their variations. Ultimately, this study help to pave the way towards a better definition of the specification of use and a better understanding of the ECP procedure.

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**Disclosure of conflict of interest:** None.

**P094**

**Successful treatment with granulocyte transfusion and early neutrophil engraftment in allogeneic transplant patients with febril neutropenia; do granulocyte transfusions help and accelerate neutrophil engraftment?**

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Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments. In this study we evaluated the effect and outcome of granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation. Between 2015–2016, five patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode. Five AML patients underwent allogeneic transplantation. Three of them transplanted from match sibling donors, one from unrelated donor, and one from (7/10) mismatch mother (haploidentical transplant). They had febrile neutropenia after transplantation, before engraftment. They had given G-CSF and antibiotics. Before the granulocyte transfusion, on the 13th–18th days of transplantation, their neutrophil counts were  $0.03\text{--}0.08 \times 10^3/\text{dL}$ . We started granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused granulocyte counts were  $3.6 \times 10^{10}$  ( $1.3\text{--}4.6 \times 10^{10}$ ) /day. After 24 h of granulocyte transfusion, mean neutrophil counts were  $0.6 \times 10^3/\text{dL}$  ( $0.4\text{--}0.8 \times 10^3/\text{dL}$ ). Neutrophil counts were  $2.1 \times 10^3/\text{dL}$ , ( $1.7\text{--}2.6 \times 10^3/\text{dL}$ ), after 48 h. After 72 h, neutrophil counts were  $3.4 \times 10^3/\text{dL}$ . ( $2.1\text{--}4.5 \times 10^3/\text{dL}$ ). After fourth days of granulocyte transfusion, neutrophil counts were normal levels ( $>0.5 \times 10^{10}/\text{dL}$ ). Conclusion: Granulocyte transfusions during neutropenic fever, helped to better-overcome febrile neutropenia periods in allogeneic transplant patients before engraftment. In addition, granulocytes transfusion also may help early neutrophil engraftment.

**Disclosure of conflict of interest:** None.

#### P095

##### Successful generation of human cytomegalovirus (HCMV)-specific T cell lines from immunodeficient patients with refractory HCMV infection or from HCMV-negative donors

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Human cytomegalovirus (HCMV) is a leading cause of morbidity and mortality in patients given allogeneic hematopoietic stem cell or solid organ transplantation, and in patients with congenital or acquired immunodeficiency. Among the patients potentially at higher risk of HCMV-related complications are HCMV-seropositive recipients of virus-seronegative HSCT donors, and HCMV-seronegative recipients of virus-seropositive solid grafts. T cell therapy may provide a unique opportunity to restore antiviral immune surveillance after transplantation, leading to clearance of infection and prevention/treatment of disease. However, priming of virus-specific responses in seronegative individuals is a challenging process. We conducted scale-up experiments to validate a method of *in vitro* culture to expand HCMV-specific T cells from 5 HCMV-negative HLA-haploidentical HSCT donors and 5 immunosuppressed patients with relapsing/refractory HCMV infection or HCMV disease, by peripheral blood mononuclear cell (PBMC) stimulation with a pool of 15-mer peptides derived from the HCMV pp65 protein. Specificity was assessed by INF $\gamma$  secretion in a Elispot assay and by cytotoxicity in a standard 51chromium release assay. T-cell lines, that included a majority of CD4+ T lymphocytes in 4/5 donors and 4/5 patients, were successfully generated from all individuals. The T-cell lines showed INF $\gamma$  production in response to pp65 (donors: median 102 SFU/105 cells, range: 0–357; patients: median 31 SFU/105 cells, range: 4–120;  $P = \text{ns}$ ), and, although the response in

patients was generally lower than in HCMV-negative healthy donors, the difference was not statistically significant. All T-cell lines presented specific cytotoxic activity against phytohemagglutinin blast target cells pulsed with pp65 peptides (donors: median 9%, range: 3–26; patients: median 14%, range: 9–48; at an effector to target ratio of 10:1), or with HCMV lysate (donors: median 20%, range: 5–31; patients: median 10%, range: 2–18; at an effector to target ratio of 10:1) with only 1/5 donors showing residual alloreactivity. The three T-cell lines showing lower INF $\gamma$  production (2 donors and 1 patient) showed high specific cytotoxic activity (31%, 10% and 19% lysis at an effector to target ratio of 10:1, respectively). Our data indicate that HCMV-specific T-cell lines with an efficient *in vitro* antiviral response and, for donor-derived cultures, low/undetectable alloreactivity against recipient targets, may be expanded from PBMC of HCMV-seronegative haplo HSCT donors and immunosuppressed patients after stimulation with HCMV pp65 protein-derived peptides. The efficacy of these T cells for pre-emptive or curative treatment of HCMV-related complications remains to be evaluated in clinical trials.

**Disclosure of conflict of interest:** None.

#### P096

##### The effect of extracorporeal photopheresis on T-cell response in graft-versus-host disease

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Extracorporeal photopheresis (ECP) is a safe and effective immunoregulatory therapy for steroid-refractory graft-versus-host disease (GvHD) but its mechanism of action is poorly understood. ECP is a non-immunosuppressive therapy whose modulating mechanism is thought to result in an increase in T-regs in the patient and in inversion of the CD4/CD8 ratio at the end of treatment. In this study, we evaluated the effect of ECP on T cell response in a cohort of steroid-refractory GvHD patients. From November 2009 to November 2016, 40 patients (28 con acute GvHD and 12 with chronic GvHD) treated with ECP in our Unit, were retrospectively evaluated. Patient characteristics are shown in Table 1. We performed an 'off-line' system ECP using a cell separator (Spectra Optia, Teruno BCT) for the CMN apheresis; after 8-methoxypsoralen was added, the product was photoinactivated in the Ultraviolet A irradiator (UVAMATIC-G1, Macopharma). ECP procedures were performed for two consecutive days, initially weekly (aGvHD), or every two weeks (cGvHD) and afterwards monthly according to clinical response. Clinical response was assessed according to Greinix *et al.* criteria. Blood samples were drawn from the patients at the beginning and in the end of ECP treatment. Lymphocyte Subpopulations (LP) CD3+, CD3+CD4+, CD3+CD8+, CD19+ and NK were studied using 5 color multiparameter flow cytometry (FC500 and Navios, Beckman Coulter). The difference between LP at the beginning and at the end of the ECP was analyzed with non-parametric tests (*U* Mann–Whitney). Statistic analysis was performed using SPSS software, version 21.0 program.  $P < 0.05$  was considered significant. In the overall analysis, all LP increased at the end of treatment. The highest increase occurred in Lymphocyte B CD19+ (pre-ECP  $19 \times 10^3/\text{mL}$ , IQR:1–150, post-ECP  $44 \times 10^3/\text{mL}$ , IQR:1–168,  $P = 0.3$ ) and in NK (pre-ECP  $78 \times 10^3/\text{mL}$ , IQR:29–133, post-ECP  $109 \times 10^3/\text{mL}$ , IQR: 48.4–171,  $P = 0.4$ ). The CD4/CD8 ratio at the start was 0.52 (IQR 0.3–1.3) and in the end 0.56 (IQR 0.31–1.2)  $P = 0.9$  (Table 2). Similar results were obtained when the analysis was performed on responders vs non responders to ECP and when the analysis was done by separating patients into acute GvHD and chronic GvHD.

[P096]

	TOTAL (n=40)		
	PRE-ECP	POST-ECP	p
WBC x10 <sup>6</sup> /ml	5800(3000-8650)	4800(1750-6650)	0.4
CD3x10 <sup>6</sup> /ml	269(97.5-852)	538(221-945)	0.9
CD3CD4x10 <sup>6</sup> /ml	112(27.3-384)	208(61-352)	0.9
CD3CD8x10 <sup>6</sup> /ml	116(37.5-432)	276(81-616)	0.8
Ratio	0.52(0.3-1.3)	0.56(0.31-1.2)	0.9
CD19x10 <sup>6</sup> /ml	19(1-150)	44(1-168)	0.3
NKx10 <sup>6</sup> /ml	78(29-133)	109(48.4-171)	0.4

ECP is a therapy that does not cause immunosuppression as measured by SL quantification, a most important feature for this type of patients. Our data do not agree with the literature regarding the normalization of CD4/CD8 ratio in responders. Although we did not determine LT regulators, there must be other mechanisms of action of ECP.

	aGVHD (%)	cGVHD (%)
Number	28	12
Female/Male	10/18	5/7
Age, years*	41(33-52)	45(38-54)
Weight (kg)*	62(50-80)	55(45-63)
Diagnosis		
AML	7(25)	4(33.3)
ALL	5(18)	3(25)
MDS	5(18)	1(8.4)
Others (NHL, MM, MF)	11(39)	4(33.3)
State previous HSCT		
CR 1 MRD	8(28)	5(41.3)
CR 2 MRD	1(4)	2(17)
MRD +	4(14)	1(8.4)
Visible Disease	15(54)	4(33.3)
HSCT source		
RD	9(32)	7(58)
URD	3(10)	3(25)
Haplo	15(54)	2(17)
Dual UCB	1(4)	0
Female donor and male recipient	13(46)	4(33.3)
Sources of Stem cells		
Peripheral Blood	24(86)	11(91.6)
Marrow	3(10)	1(8.4)
UCB	1(4)	0
GVHD prophylaxis		
Csa-MTX	12(42)	8(66.6)
Csa-CFM-MMF	15(54)	3(25)
Others	1(4)	1(8.4)
Previous treatment lines pre ECP		
1	10	6
2	15	4
3	3	2
Days between onset of GVHD and ECP *	30 (16-60)	150 (30-255)
No of procedures ECP*	13(8-20)	22(12-31)
ECP procedures to clinical response	3(2-4)	4 (2-3)
Clinical response after ECP (Days) *	8 (2-12)	14 (8-34)
Steroids decrease after ECP (Days) *	8 (2-12)	20 (7-30)
Duration in days ECP*	59(25-115)	112(71-166)

\*Dates: median (range). AML:Acute Myeloid Leukemia; LAL:Acute Lymphoid Leukemia; MDS:myelodysplastic syndrome; NHL:Non Hodgkin Lymphoma; MF: myelofibrosis; MM: Multiple Myeloma; RD: Related Donor; URD: Unrelated Donor; UCB: umbilical cord blood; MRD: Minimal Residual Disease;GVHD: Graft versus Host Disease

Disclosure of conflict of interest: None.

### P097

#### Therapeutic efficacy of bone-marrow-derived mesenchymal stem cells on acute anthracyclines cardiotoxicity enhanced by microRNA-21-mediated regulation of apoptosis and angiogenesis

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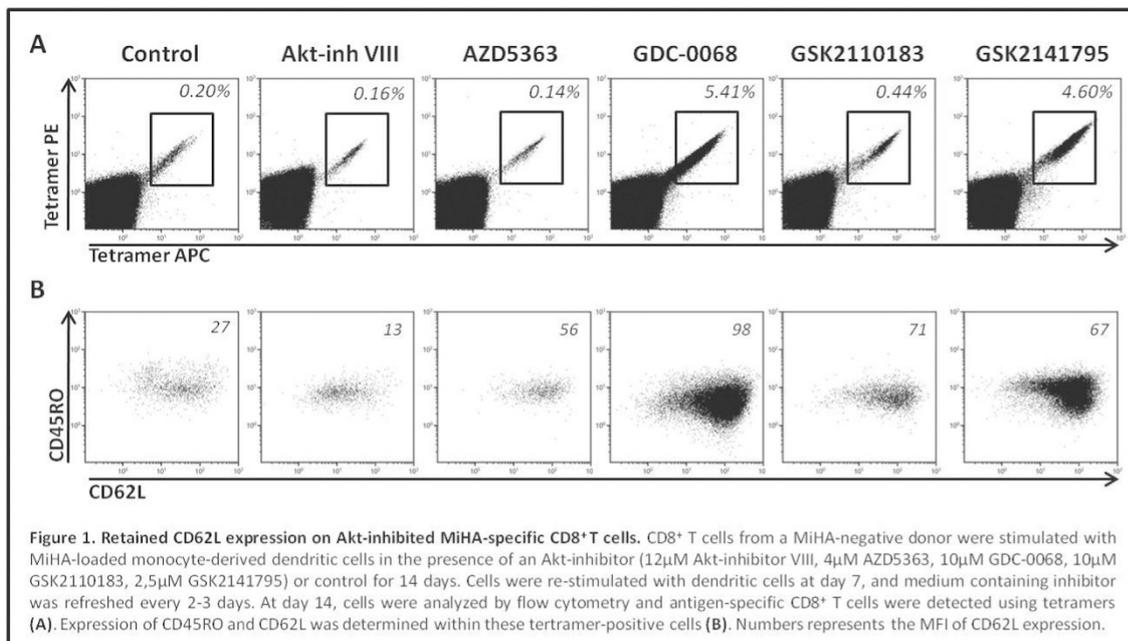
Anthracycline-induced cardiotoxicity (AIC) is irreversible, which has limited the use of this anthracycline in cancer chemotherapy. To explore the therapeutic effect and its possible mechanism of bone marrow derived mesenchymal stem cells (BMSCs) on cardiac damage induced by anthracyclines in a rat model. Study selects SD rats aged 2-3 weeks to isolate and culture BMSCs, and flow cytometry was used for phenotypic identification of BMSCs. 48 female SD rats were first randomly divided into 6 groups: the sham control, BMSCs control, 4.0 mg/kg daunorubicin (DNR), DNR with BMSCs, DNR with Dexrazoxane (DZR), DNR with BMSCs and DZR. Left ventricular (LV) function before, during and after chemotherapy were assessed by Echocardiography. At the end of 4 weeks, animals were euthanized and organs were collected in 10% buffered formalin for histopathology using hematoxylin and eosin staining and immunohistochemical analysis was used to identify the cellular subpopulations that infiltrate the cardiac tissues. After the construction of microRNA-21 (miR-21)-modified BMSCs with lentiviral vector, 50 SD rats were randomly assigned into 5 groups: the normal control, the empty vector control, DNR, DNR with BMSCs, DNR with miR-21-modified BMSCs. The density of new blood vessels of rats in each group was detected by immunohistochemical method. miR-21, Bcl-2, Bax and VEGF mRNA expressions were detected by qRT-PCR. Bcl-2, Bax and VEGF, Cx43, troponin T and BNP protein expressions were detected by western blotting. All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional. An animal model of drug-induced cardiomyopathy was built in the DNR treated rats. LV ejection fraction (LVEF) and LV fractional shortening (LVFS) were significantly decreased compared to that of the sham control ( $P < 0.001$ ), and the signs of the myocyte injury (myocytolysis, vacuolization and disruption) in paralleled with the inflammatory infiltrates, marked by CD3 and HLA-DR, were observed in the DNR group, while BMSCs alone or synergistic with DZR facilitate the anthracycline-induced LV dysfunction returning to the baseline values and the recovery of myocarditis ( $P < 0.001$ ). In the miR-21-modified BMSCs transplant group, miR-21 expression, cell migration and proliferation ability were higher than that in the BMSCs and empty vector groups ( $P < 0.05$ ). The cardiac regenerative capacity of BMSCs following significant myocardial injury were further enhanced by miR-21 compared to that of the DNR group and the control groups (all  $P < 0.05$ ), revealed by the significantly higher density of new blood vessels and upregulation of VEGF expressions, during which the pro-apoptotic protein Bax were down-regulated and the anti-apoptotic protein Bcl-2 function were upregulated in the miR-21 overexpression group compared to that with the BMSCs, DNR group and the control groups (all  $P < 0.05$ ). Western blotting demonstrated that the expression of Cx43 were significantly decreased, while expressions of troponin T and BNP were significantly increased in the miR-21 overexpression group in contrast to that with the DNR group (all  $P < 0.05$ ). These results showed that BMSCs could reverse cardiac damage induced by anthracycline, and the cardioprotective efficacy was further enhanced by miRNA-21-mediated regulation of apoptosis and angiogenesis.

Disclosure of conflict of interest: None.

### P098

#### Towards adoptive transfer of Akt-inhibited early memory CD8+ T cells for a superior graft-versus-leukemia effect

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Effective adoptive T cell therapy against cancer is dependent on long-lived tumor-specific stem cell-like T cells with the ability to self-renew and differentiate into potent effector cells. However, current protocols for *ex vivo* generation of tumor-specific CD8<sup>+</sup> T cells result in terminally differentiated effector T cells. It was found that minor histocompatibility antigen (MiHA)-specific CD8<sup>+</sup> T cells with an early memory-like phenotype and long-lived memory transcription profile could be expanded from naive precursors using Akt-inhibitor VIII1. Importantly, these Akt-inhibited tumor-specific CD8<sup>+</sup> T cells showed a superior expansion capacity and anti-tumor effect multiple myeloma bearing mice. For the clinical exploitation of *ex vivo* generated Akt-inhibited tumor-specific CD8<sup>+</sup> T cells, we tested the effect of potential clinical grade Akt-inhibitors AZD5363, GDC0068, GSK2110183, GSK2141795, MK2206 and Triciribine in polyclonal stimulations, allogeneic mixed lymphocyte reactions (MLR), and antigen-specific T cell assays. Polyclonal stimulation with anti-CD3/CD28 beads on CD8 naive T cells was used for a first screening of the Akt-inhibitors. For all inhibitors, a dose dependent effect on the naive-associated receptors CCR7, CD62L and CXCR4 was observed. This had limited effect on viability, activation and proliferation except for Triciribine, which was therefore excluded for further assays. Moreover, in the MLR, treatment of naive CD8<sup>+</sup> T cells with remaining Akt-inhibitors resulted in a dose dependent effect associated with higher CCR7, CD62L, CXCR4 and CD28 expression. Furthermore, the Akt-inhibited CD8<sup>+</sup> T cell products showed a 10–25 fold increased expansion capacity upon restimulation *in vitro*. When expanding MiHA-specific CD8<sup>+</sup> T cells from the naive repertoire in the presence of one of the Akt-inhibitors, the MiHA-specific CD8<sup>+</sup> T cells showed a more early memory phenotype compared to controls. This was displayed in higher levels of the naive-associated receptor CD62L (Figure 1). In addition, these MiHA-specific CD8<sup>+</sup> T cells were shown to be functional, as antigen-specific restimulation resulted in degranulation (CD107a) and IFN- $\gamma$  production. Based on this IFN- $\gamma$  production, the Akt-inhibited antigen-specific CD8<sup>+</sup> T cells can be selected using the Cytokine Capture Assay (Miltenyi), for enriched infusion in patients suffering from hematological malignancies. Using Akt-inhibition in the generation of tumor-reactive T cells results in a more early memory tumor-specific CD8<sup>+</sup> T cell product. This adoptive immunotherapy product retains superior

proliferation capacity upon infusion, and its potential self-renewal capacity could result in a long-term anti-tumor effect in patients suffering from a hematological malignancy.

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**Disclosure of conflict of interest:** None.

#### P099

#### Use of reversible streptamer technology to isolate clinical grade regulatory T cells from frozen umbilical cord blood units for cell therapy

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While haematopoietic stem cell transplantation is a curative treatment for many haematological disorders, a major complication is graft versus host disease (GvHD). Several early phase studies have investigated using regulatory T cells (Tregs) as a cellular therapy for GvHD to reduce the use of immune suppressive agents. Of particular interest is the use of third party cryopreserved umbilical cord blood (CB) as an 'off-the-shelf' cell source. This project, part of the European Union project 'T-control', investigated using streptamer technologies to isolate Tregs from cryopreserved CB units, offering a minimally manipulated cell population without the presence of the selection antibody. CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>+</sup> cells were

magnetically separated using anti-CD25 and anti-CD4 streptamers using a single or double step procedure. The streptamers were dissociated using D-biotin. Selected cells were cultured for up to 3 weeks using clinical grade compatible protocols<sup>1</sup> with either anti-CD3/28 dynabeads (Life sciences) or anti-CD3/28 expansion beads (Miltenyi Biotec). Treg function was measured by suppression of CFSE labelled adult CD4+ cells, to soluble anti-CD3. Responses to IL-2 were assessed with STAT5 phosphoflow. Cultured cells were screened for Treg exhaustion and chemokine receptor markers to determine the stability of the Treg phenotype. Single selection resulted in a high Treg purity of CD4+ cells (mean 90%) but with CD3-contaminants (40% Tregs of CD45+ cells). Double positive selection (CD4+ then CD25+) produced higher purity (mean 80% of CD45+ cells, >90% of CD4+ cells). However, cells isolated from cryopreserved CB units could not suppress adult CD4 responses to anti-CD3, immediately post isolation. When repeated using fresh CB, suppressive function correlated with CD25 levels on the FOXP3+ cells. In turn, CD25 levels were related to the degree of loading of the streptamer with anti-CD25-fab. Further analysis indicated that STAT5 signalling responses to IL-2 were impaired. Despite this apparent low suppressive function, cells isolated from cryopreserved CB expanded 50–100 fold within 2 weeks and suppressive function was restored. Furthermore, the cells had a stable Treg (FOXP3+Helios+) phenotype. The streptamer technology results in an 'untouched' cell product, and allows expansion prior to clinical use. The technology also, uniquely, allows for multiple positive selections without flow sorting, and increasing purity. Despite the technique affecting suppressive function immediately post isolation, the product can be expanded 100 fold in 2–3 week culture using GMP compatible conditions, to result in a highly suppressive population. These expansions are comparable to those previously described, using similar methodology<sup>2</sup>. Streptamers have the potential (external magnet with closed system and reagent costs only) to be a more cost effective approach than clinical grade column- or sorting-based methods.

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- Disclosure of conflict of interest:** The research leading to these results is funded by the European Union's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 601722.

## Gene Therapy

### P100

#### IL-2 and IL-15 allow the generation of gamma-delta CAR-T cells with potent anti-leukaemia activity: A foundation for off-the-shelf cellular immunotherapy of haematological malignancies

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Chimeric antigen receptors (CARs) are composed of an extracellular domain-derived from a tumour-reactive monoclonal antibody, linked to one or more signalling endodomains. In early clinical trials, CD19 CAR-T cells have demonstrated impressive anti-tumour activity against different B-cell malignancies, including chronic lymphocytic leukaemia, acute lymphoblastic leukaemia (ALL) and non-Hodgkin

lymphoma. Conventional alpha-beta CAR-T cells are however HLA-restricted and could cause graft-versus-host disease (GVHD) when used across major mismatches, as expected in the highly anticipated setting of off-the-shelf CAR-T cells from third-party donors. Besides being non-HLA restricted, gamma-delta T cells possess intrinsic anti-tumour reactivity, making them attractive effectors for next-generation CAR-T cell therapies. So far, however, attempts at exploiting gamma-delta T cells in patients have been largely disappointing, possibly because of sub-optimal *ex vivo* culture conditions. The aim of our study was to optimise the generation of gamma-delta CAR-T cells and to test their anti-tumour potency both *in vitro* and *in vivo*. Starting from peripheral blood mononuclear cells of healthy donors, we stimulated gamma-delta T cells with zoledronate and IL-2/IL-15, and transduced them with retroviral vectors encoding for CD19 CARs carrying either CD28.z or 4-1BB.z signalling endodomains. We assessed anti-tumour activity *in vitro* by measuring killing, secondary expansion and cytokine production after co-culturing gamma-delta CAR-T cells with different CD19+ ALL cell lines, and *in vivo* in NSG previously engrafted with a B-ALL semi-cell line. Fourteen days after stimulation, we obtained a robust gamma-delta T-cell expansion (fold increase: median 89.9, range: 23.2–144.7) with a specific enrichment of naturally anti-tumour Vgamma2+ cells (median 92.0%, range: 83.4–94.8%). Gamma-delta T cells could be efficiently transduced with anti-CD19 CARs (median 47.6%, range: 41.1–88.8%) acquiring robust killing capacity against tumour-cell lines (average killing: 96%, range: 94.2–99.9%) and producing effector cytokines, such as IFN-gamma and TNF-alpha. These properties were more evident when using a 4-1BB.z endodomain and could not be achieved by culturing with high-dose IL-2. Following infusion in tumour-bearing NSG mice, gamma-delta CAR-T cells persisted as long as alpha-beta control CAR-T cells (1–2 weeks) and mediated equal anti-tumour effects, as demonstrated by prolonged (> 3 weeks) disease free-survival compared to mice treated with either saline or un-transduced gamma-delta T cells, without causing xenogeneic GVHD. Lack of anti-tumour effects by un-transduced gamma-delta CAR-T cells as observed in this model confirms that the natural anti-tumour reactivity of gamma-delta T cells is per se insufficient to control tumour outgrowth *in vivo*, possibly explaining the disappointing results obtained so far in clinical studies. By using IL-2/IL15 culturing, we have optimised the generation of fully functional gamma delta CAR-T cells that, being non-HLA restricted, could be used across HLA mismatches without causing GVHD. Once validated in the clinical setting, these results may constitute the foundation for off-the-shelf cellular immunotherapy of haematological malignancies, overcoming the challenges of personalised manufacturing for patients in urgent need of curing therapies.

**Disclosure of conflict of interest:** None.

### P101

#### Molecular and functional analysis of mesenchymal stromal cells isolated from the bone marrow of patients affected by beta-thalassemia

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Bone marrow (BM) contains a rare population of mesenchymal stromal cells (MSCs) that, together with endothelial cells and osteoblasts, provide a specific microenvironment to support hematopoietic stem cell (HSC) homeostasis. Beta-thalassemia (BT) is a group of hereditary blood disorders characterized by anomalies in the synthesis of  $\beta$ -chain of hemoglobin. Data on the mesenchymal compartment in BT patients (pts.) are scarce. We evaluated the effects of iron exposure on the BM niche in terms of isolation, expansion and function of MSC derived from BT BM samples. BT-MSC were also studied in the murine model of the disease. Mononuclear cells were isolated from 8

BT (age range: 5–33 years) and 6 age-matched healthy donor (HD) BM samples by density gradient centrifugation. After immunomagnetic isolation of CD34+ HSC, the remaining CD34- fraction was plated in the presence of LG-DMEM + 5% platelet lysate to obtain MSCs. Morphology, clonogenic potential (CFU-F), proliferative capacity, immunophenotype, differentiation capacity, ability to respond to iron stress were evaluated. MSCs derived from compact bone and BM of Hbbth3/+ (th3/+) BT mice were cultured and analyzed in terms of clonogenic capacity, proliferation, immunophenotype, immunofluorescence staining for Nestin on BM sections (th3/+ and wt mice). BT-MSCs showed a reduced clonogenic capacity with delay in colony formation and lower numbers of CFU-F compared to controls ( $P < 0.05$ ). We calculated longer population doubling time for BT-MSCs, which proliferated less than controls. Similarly, we observed an altered differentiation capacity into adipocytes and osteoblasts in BT- compared to HD-MSCs. Both HD- and BT-MSC expressed the canonical mesenchymal markers CD73, CD105 and CD90. On the contrary, the expression of CD146 was extremely reduced in BT-MSCs compared to the HD both by FACS and qPCR, indicating a pauperization of the most primitive stem cell pool. We analyzed the expression of genes involved in the crosstalk between MSCs and HSCs in the BM and found a reduced expression of Cxcl12, SCF and AngP1 in BT-MSC. Similarly, several genes relevant for MSC functionality (Vegf, Jag, N-Cad, VCAM, FGF2 and IL-6) were downregulated in BT-MSC compared to HD. BT- and HD-MSCs were grown in the presence of high concentration of iron to mimic the stressful physiological condition of BM microenvironment in BT pts. Both BT- and HD-MSCs inhibited the expression of transferrin (TFR1) and upregulated the expression of ferritin (FTH, FTL) in order to reduce iron uptake and store it in a less toxic form. However, only HD-MSCs massively induced the expression of HMOX1, which has a protective role as antioxidant. Preliminary evidences in the murine model confirmed pts. data in terms of reduced *in vitro* clonogenic and proliferative capacity of th3/+ MSC compared to wt controls. Histological analyses of th3/+ BM revealed an altered organization of Nestin+ MSC within BT niche. We showed a reduced percentage of CD146+ primitive cells in the stromal population of BT pts, which correlated with reduced CFU-F formation, impaired proliferation and differentiation capacity. We also observed an altered ability to respond to iron overload and a strong downregulation of genes involved in MSC functionality, altogether indicating that the BM niche is impaired in BT pts. Data on the murine model of the disease confirmed the altered function and organization of BT-MSC.

**Disclosure of conflict of interest:** None.

## New drug- and cell-based immune therapies

P102

Previously published

P103

### Donor lymphocyte infusion after haploidentical transplantation with post-transplant cyclophosphamide

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Although allogeneic hematopoietic stem cell transplantation (alloSCT) is a curative option to treat hematologic malignancies, disease recurrence remains a concern in the setting of high risk diseases. Thus, post alloSCT therapeutic strategies are needed to treat and/or prevent disease progression. In this setting,

donor lymphocytes infusion (DLI) is an option as post alloSCT immunotherapy aiming to enhance graft versus leukemia (GVL) effect. Although DLI may induce persistent remission, graft versus host disease (GVHD) is a potential complication following DLI. Because of the suspected higher incidence of GVHD in the presence of HLA mismatches, few series focused on DLI following haploidentical stem cell transplantation (HaploSCT) so far. We therefore report our experience of DLI following HaploSCT using post-transplantation cyclophosphamide (PT-Cy) platform. We included in this single center study all consecutive adult patients with hematological malignancies who received DLI after HaploSCT with PT-Cy as part of GVHD prophylaxis from 2013 to 2016 ( $n = 21$ ). Conditioning regimens were non-myeloablative (low dose TBI-based) or with reduced toxicity (various dose of busulfan according to disease and patient characteristics). Cyclosporine A and mycophenolate mofetil were given as additional GVHD prophylaxis in all cases. DLI were given at escalating doses, expressed as CD3+cells/kg, without GVHD prophylaxis, and ranged from  $1 \times 10^5$  to  $5 \times 10^7$  cells/kg. Eleven patients (52%) were transplanted for high risk disease according to the disease risk index (DRI, Armand *et al.*, blood 2015). Twelve patients (57%) received haploSCT in complete remission. 18 patients received first transplant and 3 patients their second transplant. After HaploSCT, 21 patients (median age: 56 years [range: 23–73]) received either therapeutic (treatment of hematological post transplantation relapse,  $n = 6$ ) or prophylactic ( $n = 15$ ) DLI. The median interval from HaploSCT to the first DLI was 128 days (range: 79–1011). The average number of DLI per patient was 1.8 (range, 1–3). Clinical characteristics are outlined in Table 1. Patients with AML and MDS received DLI alone ( $n = 13$ ) or in association with azacytidine ( $n = 2$ ). Patients with MM received DLI in association with Revlimid ( $n = 3$ ). Chimerism before first DLI was complete in 19 patients. 6 patients (33%) developed post DLI GVHD in a median time of 42 days (range: 30–210) with exclusively chronic features. 2 patients (9%) had severe forms of chronic GVHD. GVHD-related death occurred in 1 patient No response was achieved when DLI were given as therapeutic and 4 of 6 patients died from disease progression. Otherwise, only 3 of 16 patients who received prophylactic DLI experienced relapse. With a median follow up of 129 days, overall survival was 63%. Our study suggests that DLI following HaploSCT with PT-Cy is feasible. GVHD is frequent but with a relatively low incidence of severe forms. No response rate was achieved in the context of hematological relapse, underlining that preemptive or prophylactic strategy might be preferred. Indeed, the overall good outcome in patients receiving prophylactic DLI is promising taking into account the poor prognostic of the diseases indicated for alternative donor transplantation. Further prospective studies are needed in specific disease settings to assess the benefit for using such post alloHSCT immune-intervention.

[P103]

		Patients' characteristics (n=21)
<b>Number of patients</b>		21
<b>Age (years) [range]</b>		56 [23-73]
<b>Gender (male/female)</b>		12/9
<b>Pathology</b>		
	Acute myeloid leukemia	13 (62%)
	Myelodysplastic syndrom	3 (14%)
	Multiple myeloma	3 (14%)
	Non hodgkin lymphoma	2 (10%)
<b>Conditioning</b>		
	Non myeloablative	7 (33%)
	Reduced intensity conditioning	10 (48%)
	Myeloablative	4 (19%)
<b>Chimerism status pre-DLI (%)</b>		
	Full donor	19 (90%)
	Partial donor	2 (10%)

**Disclosure of conflict of interest:** None.

**P104**

**Dual specific cytokine-induced killer cell therapy as a treatment option for life-threatening PTLD—a case report of the Frankfurt experience**

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Infection with Epstein-Barr virus (EBV) is a frequent complication after allogeneic hematopoietic stem cell transplantation (HSCT) and besides relapse remains a significant cause of morbidity. Prolonged immunosuppression or delayed T-cell recovery may favor EBV reactivation after transplantation, which under these circumstances can lead to life-threatening lymphoproliferative disease (PTLD). Consensus is lacking on the optimal treatment of PTLD. Adoptive immunotherapies with both anti-tumor capacity and restored virus-specific cellular immunity may represent optimal treatment options especially when considered in the context of PTLD. In this case report we applied *in vitro* activated T-cells namely cytokine-induced killer (CIK) cells with dual specific cytotoxic capacities transferring both anti-cancer potential and donor T-cell memory against EBV infection for the treatment of EBV-associated PTLD which progressed to highly proliferative large b cell lymphoma during delayed T-cell recovery after allogeneic HSCT. The reported patient had received an allogeneic HSCT for secondary myelodysplastic syndrome following acute myeloid leukemia, and due to delayed T-cell recovery had developed EBV-related PTLD two months after transplantation. Treatment with Rituximab, conventional EBV-specific T-cells and wildtype CIK cells failed, therefore the patient was offered EBV-specific CIK cells on a compassionate use basis. EBV-specific CIK cells were generated from peripheral blood mononuclear donor cells. Cells were activated and expanded in the presence of IFN-γ, IL-2, anti-CD3 antibody and IL-15. On day 0 and 2 of culture an EBV peptide pool was added for additional priming. Follow-up analysis included *in vitro* and *in vivo* monitoring of EBV-specific CIK cells. With above mentioned protocol we were able to

generate CIK cells containing  $1 \times 10^4$  CD8<sup>+</sup> EBV-specific T-cells/kg body weight of the patient. Infusion of EBV-specific CIK cells resulted in rapid clearance of plasma EBV DNA level and sustained disappearance of large (vol. 27cm<sup>3</sup>) PTLD-malignant lymphoma. During one-year follow-up analysis we were able to detect EBV-specific CIK cells (CD4<sup>+</sup> and CD8<sup>+</sup>) *in vivo* by flow cytometry using specific MHC-dextramers. FACS- monitoring of the patient's blood revealed besides CD8<sup>bright</sup> T-cells also an increasing CD8<sup>dim</sup> T-cell population with a remarkable percentage of T<sub>EMRA</sub> cells within this compartment (up to 95%) indicating virus-specific T-cells. No cytokine release syndrome appeared after EBV-specific CIK cell treatment, but cytokine secretion patterns, analyzing serum of the patient, reflected cytotoxic and anti-virus capacity provided by this treatment. Cytotoxic potential, as well as T<sub>H1</sub> cell differentiation and function offered by EBV-specific CIK cell treatment were further confirmed by *in vitro* analysis. EBV-specific CIK cells revealed an 1.8-fold increased cytotoxic potential *in vitro* towards EBV peptide-loaded target cells (E:T ratio 5:1) compared with wildtype CIK cells (42.8% ± 14.4% to 22.7% ± 8.9%; *P* < 0.05; *n* = 7), while anti-leukemic efficacy was retained. Mean specific lysis of leukemia cell lines K562 and THP-1 were 32.2% and 42.8% at an E:T ratio of 20:1 when using EBV-specific CIK cells and 33.5% and 48.7% using wildtype CIK cells, respectively. EBV-specific CIK cells showed great promise for prevention of malignant disease and treatment of EBV-complications after allogeneic HSCT.

**Disclosure of conflict of interest:** None.

**P105**

**Extracorporeal photopheresis for treatment of graft versus host disease: The experience of our centre**

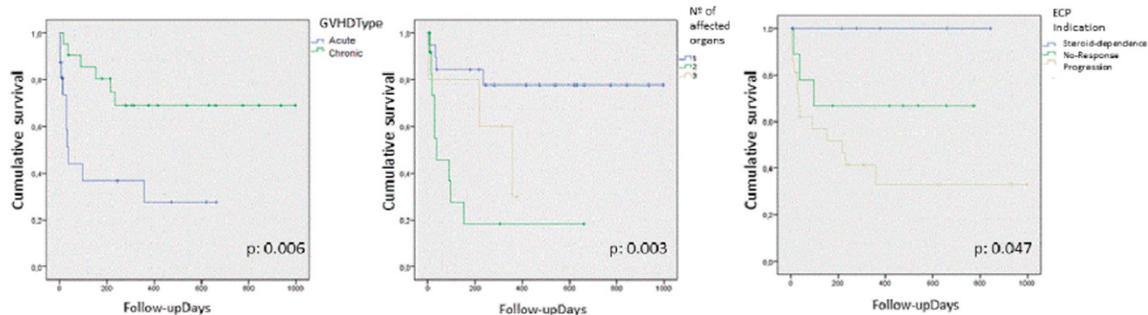
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Despite the advances in prevention and the treatment, aGVHD and cGVHD are the most common causes of morbidity and mortality of allo-SCT. Although the treatment after the first line remains unclear, extracorporeal photopheresis (ECP) is considered a good alternative. We retrospectively review patients treated with ECP for GVHD in a single center in Granada, Spain. A general descriptive overview of the sample and survival analyses (Kaplan–Meier approach with a significance level of 0.05) are provided. Thirty-seven patients were treated with ECP, 16 (43.2%) for aGVHD and 21 (56.8%) for cGVHD, showing the characteristics described in Table 1. The frequencies aGVHD severity degrees were 2: 31.25%; 3: 43.75% and 4: 25% while for cGVHD they were as follows: mild: 4.8%; moderate: 61.9% and severe: 33.3%. For 51.4% of the patients the main affected organ was the skin while more than one organ were affected in 48.6% of the cases. The mean follow-up time was 294 days (4–996), with 77.2% of the patients obtaining a response and the immunosuppression being decreased in the 62.9% of them. The complications during the process were 4 cases of catheter-associated infections and 1 relapse in the

[P105]

Characteristics of the patient	
Age (mean age in years)	42
Gender (%): male, female	67.6%, 32.4%
Diagnosis (%): acute leucemia, lymphoma, myelodysplastic syndrome, others	46%, 32.4%, 13.5%, 8.1%
Conditioning regimen (%): MAC, RIC	62.2%, 37.8%
Donor type (%): sibling, unrelated	51.4%, 48.6%
Compatibility (%): matched, mismatched	75.7%, 24.3%



hematologic disease. The global survival ratio in the follow-up was 56.8% (with 66.6%, 60.6% and 42.8% survivals in 3, 6 and 12 months, respectively). The variables significantly associated with greater survival were: type of GVHD (cGVHD), number of affected organs (an organ had to be moderately or severely affected to be included in this category) and steroid dependence as the main reason to initiate ECP (see Figure 1). There was a trend towards significance for the degree of GVHD and cutaneous involvement to be factors associated to enhanced survival ratios. Extracorporeal photopheresis is a safe treatment option for patients with GVHD, generating a response and decreasing immunosuppression in an important percentage of them. The presence of cGVHD rather than aGVHD, a lower severity degree of the condition, having a lower number of affected organs, skin as main affected organ and steroid dependence as the reason to start the ECP treatment were all factors associated with greater survival in our sample.

**Disclosure of conflict of interest:** None.

#### P106

##### **Ibrutinib as bridge therapy to allogeneic stem cell transplantation in refractory mantle cell lymphoma (MCL): A case report**

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Mantle cell lymphoma (MCL) is a heterogeneous subtype of non-Hodgkin lymphoma. Conventional treatment with immunochemotherapy followed by autologous stem cell transplantation (SCT) or intensive immunochemotherapy alone improved outcome, but the disease remains incurable with chemotherapy only. Ibrutinib was approved by FDA for refractory/relapsed MCL. No data are available about use of Ibrutinib before allogeneic stem cells transplantation (allo-Tx). We report the case of a patient refractory to chemotherapy treated with Ibrutinib as debulking therapy before allo-Tx. In June 2014, a 62 years old woman was diagnosed with MCL. The staging performed by whole body CT scan, colonoscopy, EGDS and bone marrow (BM) biopsy was conclusive for stage IVA with bulky lymph node over and below the diaphragm, BM, enteric and peripheral blood (PB) localization. The planned treatment included 3 cycles of R-CHOP, 1 cycle of High dose (HD) CY, 2 cycles of HD ARAC and autologous SCT. After completion of HD CY, the restaging showed progressive disease, with a thyroid involvement and histologic switch in a blastoid variant. Disease continued to progress even after 2 cycles HD ARAC, so we tried to control the disease with R-Bendamustine (90 mg/mq on days 1–2 of 21-d cycle), but after the first cycle the neck circumference increased. We shift to Lenalidomide (25 mg on days 1–21 of 28-d cycle) without any response after two cycles. We excluded patient from autologous SCT programme because of chemo-refractoriness and we searched matched unrelated donor because no HLA identical sibling was available. We started a therapy with Ibrutinib (560 mg/die on days 1–21 of 21-d cycle). After the first cycle we observed a rapid response with decrease of neck size and the disappearance of superficial lymphnode; we performed 6 cycles of Ibrutinib, and we reached a good partial remission with lymphnode of max 4 cm, and a BM and PB involvement of 20%. Meantime an unrelated donor with 7/8 HLA matching was identified, so in December 2015 we performed allo-Tx with reduced-intensity conditioning (Thiotepa 5 mg/kg—Fludarabine 90 mg/m<sup>2</sup>—Melphalan 100 mg/m<sup>2</sup>) and Cyclosporine and short term Methotrexate as GVHD prophylaxis. Engraftment was at day +17. In the first 100 days after allo TX she experienced a clostridium enteritis, transient CMV reactivation and acute gastrointestinal GVHD on day +60 with rapid response to steroid therapy. Main complication happened on day 100, when sudden fever and stupor, progressive to coma, occurred; subsequently Pneumococcal encephalitis was diagnosed, with positive CSF microbiological exam and two signal alteration in the right cerebral hemisphere

at MRI. The patient was treated with ampicillin and ceftriaxone with a favourable outcome. The MCL reevaluations performed at 2, 5 and 9 months showed complete remission with disappearance of all pathological lymph node and PB involvement. Currently the patient is at 1 year post ASCT, she is enrolled in a rehabilitation program and MCL is in complete remission. Our experience seems to indicate that ibrutinib is safe and can be used as bridge to Allo-tx therapy in refractory MCL. We will investigate side effects of this platform of therapy, and, given the early occurrence of pneumococcal infection, we will consider to perform capsulated bacteria vaccination before allo-Tx.

**Disclosure of conflict of interest:** None.

#### P107

##### **Inotuzumab ozogamicin and DLI can induce molecular remission in acute lymphoblastic leukemia relapse after allogeneic HSCT**

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Relapse (Rel) of ALL after allogeneic hematopoietic stem cell transplantation (HSCT) has a dismal outcome, with median survival of 4–5.5 months. Immunotherapeutic options for salvage therapy include inotuzumab ozogamicin (IO), blinatumomab (blina) and donor lymphocyte infusions (DLI). Treatment of relapsed/refractory (R/R) ALL with IO, a monoclonal anti-CD22 antibody (MAB) conjugated to calicheamicin, allows a significant proportion of patients (pt) to proceed to HSCT in molecular complete remission (moCR). After HSCT, veno-occlusive disease (VOD) of the liver is a major concern. To date, the combination of IO and DLI has not been reported. Pt 1 was diagnosed with common(c)-ALL in 2004 aged 17 and treated as summarized in Table 1. Rel4 emerged in 12/15 and was treated with two cycles of blina followed by 3 × DLI, leading to MRD+ CR. Rel 5 in 3/16 was treated with 3 cycles IO and one DLI between second and third cycle until, with development of steroid-refractory aGVHD grade 2 (skin, oral mucosa and gut)in 6/16 which improved with extracorporeal photopheresis. 27 days after the last IO administration, in the context of a gram-negative sepsis, the pt. developed hyperbilirubinemia and ascites (transudate, no malignant cells). Imaging showed liver fibrosis and portal hypertension. Due to thrombocytopenia and suspected GvHD, liver biopsy was postponed until 9/16, when after clinical improvement histology was diagnostic for VOD. In 11/16 severe, late onset VOD (revised EBMT criteria, Mohty *et al.*, BMT 2016) recurred. Defibrotide 25 mg/kg BW daily was administered for two weeks, resulting in CR of the VOD. To date, the pt remains in moCR.

#### [P107]

Table 1: Treatment of pt 1 \*CD3<sup>+</sup>cells/kg BW

Time	Disease stage	Chemo/MAB therapy	Cellular therapy	Best response
2004	c-ALL	Chemo (ALL BFM 2000)		CR
1/13	Rel1	Chemo (IDA-FlaC)	1 <sup>st</sup> MUD-HSCT (TBI 12Gy, CY)	CR
10/14	Rel2	Chemo (GMALL 07/2013)		CRi
4/15	Rel3	Blina, 2 cycles	2 <sup>nd</sup> MUD-HSCT (fludarabine, melphalan)	MoICR
12/15	Rel4	Blina 1 12/15; II 1/16	DLI I 11/15 0.5x10 <sup>6</sup> *; II 1/16 0.12x10 <sup>6</sup> *; III 2/16 0.23x10 <sup>6</sup> *; IV 3/16 1x10 <sup>6</sup> *	MRD+ CR
3/16	Rel5 (knee, local LN)	IO I 4/16; II 5/16; III 6/16	DLI V 5/16 2x10 <sup>6</sup> *	MoICR

Pt 2 was diagnosed with c-ALL in 2014 and received a MUD-HSCT (TBI 8Gy, cyclophosphamide) in 1/15 due to persistend MRD. Following early rel 6/15, 2 cycles of blina led to MRD+ CR, for which a 2nd HSCT from a haploidentical family donor (busulfan, thiotepa, fludarabine) was performed in 10/15. MoICR lasted 3 months and rel3 was treated with 3 cycles of

weekly IO followed by one DLI ( $5 \times 10^{-4}$  CD3+ cells/kg), resulting in MRD+ CR and complete donor chimerism. Five weeks after the last IO cycle, the pt was admitted with ascites, hyperbilirubinemia and reduced general condition. VOD was suspected, but diagnostic paracentesis revealed malignant ascites demonstrating fatal progressive disease. Conclusion. Our data suggest that the sequential use of IO and DLI is feasible even for heavily pretreated patients with R/R ALL after HSCT and can induce molecular remissions. We observed an unusual case of late onset, severe VOD responding to

defibrotide and one ALL relapse manifesting itself with ascites in our patients. We therefore suggest close monitoring of liver function tests in the setting of this therapy and extensive diagnostic work-up for any developing liver abnormalities or ascites.

**Disclosure of conflict of interest:** NG: Advisory Board (Pfizer, Amgen); Research Support (Amgen); GB: Honoraria (Amgen).

[P108]

**Table 1: Inclusion/exclusion criteria**

Inclusion criteria	Exclusion criteria
<p>1. Any of the following hematologic malignancies:</p> <ul style="list-style-type: none"> <li>- Acute myeloid leukemia (AML) in first cytomorphological remission with Disease Risk Index (DRI) intermediate or above, or in second or higher cytomorphological remission</li> <li>- Acute lymphoblastic leukemia (ALL) in first or higher remission</li> <li>- Myelodysplastic syndrome (MDS): transfusion-dependent (requiring at least one transfusion per month), or intermediate or higher IPSS-R risk group</li> </ul> <p>2. Clinical justification of allogeneic stem cell transplantation where a suitable HLA matched sibling or unrelated donor is unavailable in a timely manner.</p> <p>3. Availability of a related haploidentical donor with <math>\geq 4/8</math> but <math>&lt; 8/8</math>, or <math>\geq 5/10</math> but <math>&lt; 10/10</math> matches at the HLA-A, -B, -C, -DRB1, and/or -DQB1 loci, as determined by high resolution HLA-typing</p> <p>4. Karnofsky Performance Status (KPS) <math>\geq 70\%</math></p> <p>5. Male or female, age <math>\geq 18</math> years and <math>\leq 70</math> years. Patients aged <math>\geq 65</math> years must have a Sorror score <math>\leq 3</math></p> <p>6. Availability of a donor aged <math>\geq 16</math> years and <math>\leq 75</math> years who is eligible according to local requirements and regulations</p>	<p>1. Availability of a suitable fully matched related or unrelated donor in a donor search</p> <p>2. Prior allogeneic hematopoietic stem cell transplantation</p> <p>3. Diffusing capacity for carbon monoxide (DLCO) <math>&lt; 50\%</math> predicted</p> <p>4. Left ventricular ejection fraction <math>&lt; 50\%</math> (evaluated by echocardiogram or MUGA)</p> <p>5. AST and/or ALT <math>&gt; 2.5 \times</math> ULN (CTCAE grade 2)</p> <p>6. Bilirubin <math>&gt; 1.5 \times</math> ULN (CTCAE grade 2)</p> <p>7. Creatinine clearance <math>&lt; 50</math> ml/min (calculated or measured)</p> <p>8. Positive pregnancy test or breastfeeding of patient or donor (women of childbearing age only)</p> <p>9. Estimated probability of surviving less than 3 months</p> <p>10. Known allergy to any of the components of ATIR101 (e.g., dimethyl sulfoxide)</p> <p>11. Known hypersensitivity to cyclophosphamide or any of its metabolites</p> <p>12. Known presence of HLA antibodies against the non-shared donor haplotype</p> <p>13. Positive HIV test</p> <p>14. Positive CMV test of the patient and negative CMV test of the donor</p> <p>15. Positive viral test of the donor for HIV-1, HIV-2, HBV, HCV, Treponema pallidum, HTLV-1 (if tested), HTLV-2 (if tested), WNV (if tested), or Zika virus (if tested)</p>

P108

**Introduction of the HATCHY study: A Phase III, multicenter, randomised controlled study to compare safety and efficacy of a haploidentical HSCT and adjunctive treatment with ATIR101 with post-transplant cyclophosphamide in patients with a hematologic malignancies**

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While allogeneic hematopoietic stem cell transplantation from matched related and unrelated donors has become a standard of care treatment for patients with hematological malignancies, transplantations from mismatched or haploidentical family donors remain challenging. Currently T-replete and T-deplete transplantation strategies are applied aiming to improve the outcome after haploidentical transplantation. Despite high rates of relapse many centers regard post-transplant cyclophosphamide, a T-replete strategy, as a standard of care approach. We have developed a T-depleted transplant approach where donor lymphocytes selectively depleted of alloreactive T-cells (ATIR101) using TH9402, arhodamide-like dye, are infused after CD34-selected haploidentical HSCT, to overcome the challenges of infectious complications, GvHD and relapse. In phase I (CR-AIR-001) we have demonstrated safe infusion of these lymphocytes at doses up to  $2 \times 10^6$  viable T-cells/kg. Recently, we reported a promising 1-year GRFS was 57% from a phase II trial (ASH 2016), that is awaiting final results soon. Here, we introduce a randomized, multicenter phase 3 study (CR-AIR-009), where 200 patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or myelodysplastic syndrome (MDS) are planned to undergo a haploidentical HSCT with either a T-cell depleted graft and adjunctive treatment with ATIR101, or with a T-cell repleted graft and use of post-transplant cyclophosphamide. Inclusion and exclusion criteria are listed in Table 1. All patients will undergo myeloablative conditioning consisting of either TBI (12 Gy) or melphalan/busulfan, in combination with thiotepa and fludarabine. Patients in the ATIR101 study group will receive ATG (2.5 mg/kg once daily for 4 days) during conditioning and ATIR101 infusion at a dose of  $2 \times 10^6$  viable T-cells/kg is given between 28 and 32 days after the HSCT. Patients in the PTCy group will receive cyclophosphamide (50/mg/kg) on day 3 and 4 (or 5) with subsequent use of immune suppression up to 6 months post-HSCT. The primary endpoint of the study is GVHD-free, relapse-free survival (GRFS). GRFS is defined as time from randomization until grade III/IV acute graft-versus-host disease (GVHD), chronic GVHD requiring systemic immunosuppressive treatment, disease relapse, or death, whichever occurs first. This endpoint captures both safety and efficacy. Additional secondary endpoints are overall survival (OS), progression-free survival (PFS), relapse-related mortality (RRM) and transplant-related mortality (TRM). Patients are planned to be randomized in study centers in Europe and North America. A number of 40–50 sites are planned to participate in the study. Enrolment is expected to continue until mid-2018 with initial results being available first half 2019. Results of this study will determine which transplant regimen provides most clinical benefit in haploidentical donor transplantation, with the promise of an effective regimen without the use of post-transplant immune suppression.

**Disclosure of conflict of interest:** JR is an employee of Kiadis Pharma.

## Non-hematopoietic stem cells

P109

**Interaction of multipotent mesenchymal stromal cells and lymphocytes**

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Multipotent mesenchymal stromal cells (MSCs) are used for prevention and treatment of graft versus host disease after allogeneic hematopoietic stem cells transplantation due to their immunomodulatory properties. MSCs fate *in vivo* after infusion is unknown. The aim of this study was to analyze the changes in MSCs and allogeneic lymphocytes properties when co-cultured *in vitro* to simulate their interactions *in vivo*. The bone marrow from 13 donors (7 male and 6 female aged 22–62 years, median 27 years) was used. MSCs were co-cultured with allogeneic lymphocytes in a ratio of about 1:10 for 4 days and their basic properties were analyzed over time. Lymphocytes were activated by adding to the culture medium 5 mg/mL of PHA (PHA-lymphocytes). Some MSCs were treated for 4 h with 500 U/mL IFN $\gamma$  ( $\gamma$ MSCs). Determination of gene expression levels was performed by reverse transcription polymerase chain reaction in real time (modification of the Taq-Man) and of antigen expression on MSCs and lymphocytes by flow cytometry. Significant reduction in the proportion of viable cells was observed in MSCs co-cultured with PHA-lymphocytes. In  $\gamma$ MSC co-cultured with PHA-lymphocytes no reduction in the proportion of living cells was revealed. This indicates the sensitization of MSCs by IFN $\gamma$  to factors secreted by PHA-lymphocytes. In MSCs co-cultured with PHA-lymphocytes and lymphocytes mean fluorescent signal intensity level (MFI) of CD90 gradually decreased. IFN $\gamma$  treatment and co-cultivation with lymphocytes led to significant increase of HLA-DR MFI on MSCs. Co-cultivation with lymphocytes increase the HLA-DR MFI on MSCs much stronger than IFN $\gamma$  treatment. Relative expression level (REL) of IDO1 gene increased dramatically in both MSCs and  $\gamma$ MSCs when co-cultured with lymphocytes. At a day 1 in  $\gamma$ MSCs REL of IDO1 increased 500 fold, and then gradually declined. In MSCs co-cultured with lymphocytes IL-6 REL increased almost 20-fold and then decreased 2-fold at the fourth day. The CSF1 REL in  $\gamma$ MSCs showed twofold increases, upon incubation MSCs and  $\gamma$ MSCs with lymphocytes CSF1 REL increased fourfold and sevenfold, respectively. Co-culture of MSC and  $\gamma$ MSCs with lymphocytes led to decrease in the proportion of CD25, CD38, CD69, HLA-DR, and PD-1 positive cells (both CD4 and CD8) after one day, compared with PHA-lymphocytes without MSCs. Proportion of CD25+, HLA-DR+ and PD-1+ cells also decreased after 4 days of co-culturing with MSC or  $\gamma$ MSCs (compared with PHA-lymphocytes without MSCs), but anyway number of activated cells increased 1.2–3 folds compared with first day. Number of CD69+ lymphocytes after 4 days of co-culturing with MSCs or  $\gamma$ MSCs did not vary significantly from control and decreased in comparison with first day. Main inhibition of activated lymphocytes by co-culturing with MSCs occurs during the first day of their interaction, and then the inhibition became less effective, moreover in MSCs decreased the REL of the main immunomodulating factors, and most of them were eliminated. MSCs treatment with IFN $\gamma$  resulted in improved survival and resistance of these cells to lymphocytes action. The results indicate that the effect of MSCs injected intravenously to patients is limited to several days.

**Disclosure of conflict of interest:** None.

## Regenerative medicine

### P111 Autologous adipose-derived mesenchymal stem cells embedded in platelet-rich fibrin for skin tissue engineering and wound healing

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Autologous adipose-derived mesenchymal stem cells (ADMSCs) embedded in platelet-rich fibrin (PRF) promote healing in different types of wounds. By avoiding the needle-related complications, PRF-embedded autologous ADMSCs graft provides a new effective stem cell-based therapeutic strategy for wound healing. Adult male (Age  $\leq 75$ yo) were equally divided ( $n=5$  per group) into group 1 (PRF only), and group 2 (PRF+ADMSCs). Regular dressing (without any agent) was used for both groups with a frequency of changing every 3 days. PRF with or without ADMSCs was patched on the wound (Maximum surface area  $77\text{ cm}^2$ ). All patients were followed up until complete healing. A complete healing was noted in both groups; however, the healing in group 1 was very slow (after 10 weeks), compared to a quicker one in group 2 (after 6 weeks). Control of the moisture was very well noted in group 2, less in group 1. Group 1 showed a lot of exudates on the wound; less exudate in group 2 were noted. Infections were absent. Both groups had a colonized wound. Signs of inflammation were very well noted in group 1; no signs of inflammation in group 2. ADMSCs embedded in PRF offered rapid wound healing responses than PRF alone.

**Keywords:** Mesenchymal stem cells, platelet-rich fibrin, engineered tissue wound healing

**Disclosure of conflict of interest:** None.

## Stem cell source

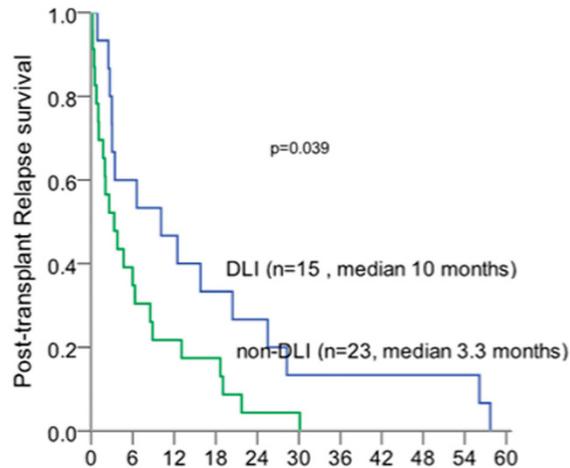
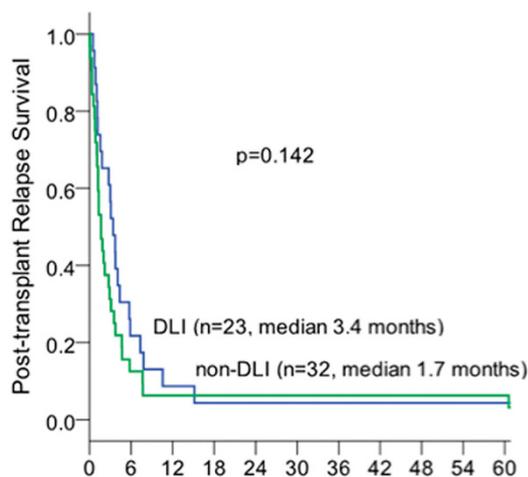
### P112 Additionally cryopreserved G-CSF primed PBSC can substitute the second transplantation for the patients with acute leukemia who lately relapsed after hematopoietic stem cell transplantation

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Although allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative therapy for acute leukemia, survival outcomes of the patients relapsed after transplantation remains poor with high early mortality and a small percentage of second remission. This study evaluated the efficacy of DLI using G-CSF primed PBSC additionally cryopreserved for the patients who relapsed after allo-HCT. We retrospectively reviewed the medical records of the 255 patients who received allo-HCT for acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL) between 1998 and 2013 in Kyungpook National University Hospital. Among the 93 patients who had relapsed after allo-HCT, the 39 patients received DLI using the additionally harvested cells. At the time of harvest for the first HCT, collecting targeted PBSCs (greater than  $6 \times 10^6/\text{kg}$  CD34+ cells) allowed us to cryopreserve surplus PBSCs, including CD3+ cells with dimethylsulfoxide in a nitrogen tank. Then, we analyzed the efficacy of DLI for the patients who were classified into early relapse or late relapse group by the median time of relapse after transplant. The median age at transplant was 38.5 years (range 15–68 years) and male was 111 patients (44.4%). Primary diseases for allo-HCT were AML/MDS ( $n=175$ , 70%) and ALL ( $n=75$ , 30%). One hundred forty three patients (57.2%) were in CR1 (complete remission), 25 (10%) in further CR, and 87 (33.2%) in relapsed and refractory status. One hundred seventy one patients (68.4%) received myeloablative conditioning regimen. The median dose of CD34+ cell was  $5.21 \times 10^5/\text{kg}$  (range:  $1 \sim 20.6 \times 10^6/\text{kg}$ ). Almost 95% of patients achieved the neutrophil engraftment with a median time of 13 days (range: 9–24days). The 1-year overall survival (OS), relapse free survival (RFS), non-relapse mortality (NRM) and graft-versus-host disease (GVHD)-free/relapse-free survival (GRFS) since HCT was  $55.3 \pm 3.1\%$ ,  $66.0 \pm 3.2\%$ ,  $28.2 \pm 0.3\%$ , and  $32.9 \pm 3.1\%$ , respectively. There was no significant difference according to

[P112]



the infused CD34+ cell dose (lower  $<6 \times 10^6/\text{kg}$  vs higher  $\geq 6 \times 10^6/\text{kg}$ ). The incidence of chronic GVHD was more frequent in higher CD34+ group (32.9% vs 48.2%,  $P=0.042$ ). Median time from HCT to relapse was 139 days (range: 21 ~ 1801 days). After relapse, 46 patients (49.4%) were treated with salvage chemotherapy, 9 patients (9.6%) with second allo-HCT, and 38 patients (40.8%) with DLI. The median number of CD3+T cell was  $2.95 \times 10^7/\text{kg}$  (range:  $0.1 \sim 5.43 \times 10^7/\text{kg}$ ). Fourteen patients (23.6%) achieved DLI induced CR, 20 patients progressed, and 6 patients were not evaluable for response. DLI induced acute GVHD was observed in 24 patients (63.1%) and chronic GVHD developed in 4 patients (10.5%). In late relapse group, the 1-year OS since post-transplant relapse was significantly higher in DLI group than non-DLI group (Figure 1,  $53.4 \pm 7.4\%$  and  $26.7 \pm 7.4\%$ ,  $P=0.039$ ) but, early relapse group had no difference. The patients treated with DLI showed significantly survival benefit in late relapse group (median 286 days vs 103 days,  $P=0.002$ ). The incidence of DLI-induced GVHD does not differ between two groups. DLI for the patients who lately relapsed after allo-HCT can be a feasible and an effective option in terms of response, donor convenience and its cost. In the late relapse group, G-CSF primed DLI may replace second transplantation. **Disclosure of conflict of interest:** None.

### P113

#### Cord blood transplantation—19 years of experience

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Allogeneic haematopoietic stem cell transplantation (HSCT) is a well-established treatment for patients with malignant and non-malignant haematological disorders. Cord blood transplantation (CBT) has extended the availability of HSCT to patients that would not otherwise be eligible for this curative approach because of the lack of human leucocyte antigen (HLA) identical donor. The aim of this retrospective study was to analyse and characterize 19 years of CBT activity in our institution (1996–2015). We examined patient electronic files and created a database in Excel to register cord blood unit (CBU) parameters and patient characteristics. After thawing, CBU was washed/diluted with validated procedures; the cellular content was evaluated by immunophenotyping and followed ISHAGE recommendations; sterility was assessed by bacterial/fungal cultures, viability by Trypan blue exclusion assay and functionality by clonogenic capacity. The transfusion of blood products after transplant was quantified and the hematological recovery (HR) established using CIBMTR criteria. Correlation between continuous variables was assessed with Spearman coefficient. Overall survival (OS) was determined according to cellular content and HLA-disparity by the Kaplan–Meier estimator. Survival between groups was compared using the log-rank test. A total of 59 CBU were administered to 57 patients (Table 1): 30/57 female, the main diagnosis was acute

leukaemia (35/57). A sibling CBU was used for 3 patients; the unrelated were imported from Europe (66%), USA (32%) and Oceania (2%). HLA-matching was 6/6, 5/6, 4/6 and 3/6 for 8, 21, 15 and 6 patients, respectively; 47% were ABO-identical. After thawing, 95% were washed and presented no microbial growth. The majority of patients were submitted to a busulfan-based myeloablative conditioning regimen; graft versus host disease prophylaxis was performed with a calmodulin inhibitor+mycophenolate mofetil. Complete and mixed chimerism was verified in 42% and 7% of patients; 5% had graft failure; the rest were unknown results (46%). At the moment, we reported 27 patients alive (22 in complete remission, 5 with evidence of disease relapse) and 30 dead at a median of 6 (0.4–198) months after CBT; the most frequent cause (43%) was recurrence of the initial clinical condition. The correlation between Nucleated cells (NC) and CD34+ cells per kg/HR ( $P=0.277$ ;  $P=0.123$ ) and number of CD34+ cells per kg/OS ( $P=0.123$ ) was not statistically significant. However, the engraftment and OS was associated with HLA-mismatch ( $P=0.025$ ;  $P=0.012$ ) and OS was related to NC per kg ( $P=0.012$ ). Our clinical results suggest that despite increased HLA disparity, UCB offers promising results. UCBT is feasible in patients when the unit contains a high number of cells. There are several strategies for the future, related to CBU expansion and homing techniques, nurturing procedures, selection of optimal CBU unit and enhancement of immune recovery, in order to improve the application of CBU.

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**Disclosure of conflict of interest:** None.

### P114

#### Cord blood transplantation supported by unrelated donor progenitor cells for patients without haplo-identical relatives

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For pts with hematological malignancies without matching related or unrelated donors, we have utilized haplocord transplants, combining a single umbilical cord blood graft with haplo-identical cells from a first or second degree family member. But among 43 searches over the past year, nine lacked appropriate haplo-identical donors. For these pts we have used partially matched unrelated donors as a source of CD34 selected progenitor cells. Median age was 56 (48–71). Seven had AML, 1 ALL and 1 Myeloma with ESRD. Conditioning consisted of fludarabine Melphalan in 1, and fludarabine Melphalan TBI 400 in 8 patients. Eight patients received GVHD prophylaxis with tacrolimus and MMF. The myeloma patient

[P113]

Table 1 – Median (range) of transplant data

Cord Blood parameters		Patient characteristics	
Cryopreservation period (months)	55 (3-130)	Age (years)	5 (0.4-50)
Cellular Viability (%)	95 (50-99)	Weight (kg)	18.4 (6-77)
Nucleated cells ( $\times 10^7/\text{kg}$ )	5.9 (0.5 – 33.1)	RBC transfusion (number of units)	9 (0-94)
CD34+ cells ( $\times 10^5/\text{kg}$ )	1.8 (0.1-47.1)	Platelets transfusion (number of units)	23 (3-184)
CFU-GM ( $\times 10^4/\text{kg}$ )	7 (1.2-100)	Neutrophils $\geq 0.5 \times 10^9/\text{L}$ (days)	19 (12-114)
Cellular recovery (%)	80 (26-100)	Platelets $\geq 20 \times 10^9/\text{L}$ (days)	37 (12-141)

received sirolimus and MMF. Median time to neutrophil and platelet engraftment was 10 days (9–13) and 18 days (17–124), respectively. One patient died from parainfluenza pneumonia (d111), one patient from PTLD (d 203), one patient from late pulmonary VOD (d379), and one patients from relapse (d89). With a median follow up for survivors of 17 months, one year survival is 64%. Three patients had grade 2–3 GVHD and none of the survivors have chronic GVHD. Though unrelated donor chimerism was dominant early after transplant and contributed to early count recovery, definitive engraftment was dominated by UCB chimerism in all but one patient. Conclusion: Among older adult patients with hematological malignancies, ~20% lack haplo-identical relatives. For these patients, double or single UCB transplant is challenging because of delayed engraftment. CD34 selected partially matched grafts from unrelated donors hasten hematopoietic recovery and are over time outcompeted by UCB grafts which provide robust hematopoiesis with low risk of chronic GVHD. The combination of mismatched unrelated hematopoietic progenitors and UCB grafts provides an attractive alternative for older patients lacking HLA-identical donors or haplo-identical relatives. In planned trials, mismatched donors may be selected based on KIR type to further enhance GVL effects. **Disclosure of conflict of interest:** Partially supported by Miltenyi Biotec.

### P115

#### Early withdrawal of immunosuppressive therapy in haploidentical bone marrow transplantation with post-transplantation cyclophosphamide increases chronic GvHD and can reduce relapses

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Haploidentical stem cell transplantation (Haplo-SCT) is an attractive option for patients who do not have an HLA-matched donor. Historically it has been associated with high rates of graft rejection, relapse and low incidence of Graft versus Host Disease (GvHD). To decrease these issues we have considered the use of primed bone marrow as stem cell source, early withdrawal of immunosuppressive therapy and the use of donor lymphocytes infusions (DLI) in haplo-SCT with high-dose post-transplantation cyclophosphamide (PTCy) as main GvHD prophylaxis. To analyse our incidence of acute and chronic GvHD and overall survival (OS) in patients with Haplo-SCT with short course of immunosuppressive therapy. We retrospectively analyzed a cohort of 28 patients who underwent haplo-SCT with primed bone marrow as stem cell source, between years 2012 and 2016 in our centre. GvHD prophylaxis consisted in PTCy (50 mgr/kg on days +3 and +4) and tacrolimus plus mycophenolate (MMF) from day +5 as recommended by Baltimore group. MMF was stopped on day +28. Tacrolimus was tapered off from day +50 with withdrawal on day +120 in patients without GvHD or with active disease. DLI were considered if mixed chimerism, relapse or disease progression appeared. The characteristics of the patients are shown in Table 1. **Results:** There was no primary graft failure. Eight of 27 patients (29.6%) developed aGvHD (grade II–IV) and it was severe (grade III–IV) in 2 patients (7.4%). Cutaneous aGvHD location was the most common presentation (9 patients (33%)) and it was associated with intestinal GvHD in 2 patients. Twenty two patients were evaluable for cGvHD. Thirteen patients (59%) developed chronic GVHD that was mild, moderate and severe in: 8 (36.3%), 3 (13.6%) and 2(9%) patients, respectively. The median time of onset cGVHD was 6 months (range: 3–33) and it was related with previous withdrawal of the immunosuppression in 5 (22.7%) patients, tapered off immunosuppression in 6 (27.2%) patients and DLI in 2 (9%) patients. Systemic

treatment was required in 8/13 patients but only 2 patients were treated with high doses of steroids (> 1 mg/kg/day). The median days of IS therapy in patients who developed GVHD was 172 days (range: 81–244). DLI were used in 7 (25%) patients because of: relapse/disease progression in 5(17.8%) and secondary graft failure in 2(7.1%). Two patients achieved complete remission and 2 patients developed cGHVD. The median number of DLI per patients were 2 (1–3) with a median CD3+cell of  $3 \times 10^6$ /kg (range:  $1 \times 10^5$ – $1 \times 10^7$ /kg). With a median follow-up of 12 months (range: 1–56), the estimated OS at 1 and 2 years after haplo-SCT were 74% and 52%, respectively. At the moment of this study 17 patients (60.7%) were alive, 14 patients in complete remission, 2 in partial response and 1 in progression. Eleven (39.3%) patients died due to: disease (4), infections (4), pleuropericarditis (1), hepatic veno-occlusive disease (1) and refractory GVHD. Five patients (17.8%) are without IS therapy and without GvHD symptoms. In our experience, early withdrawal of immunosuppression following haplo-SCT with primed bone marrow and post-transplantation cyclophosphamide facilitates the development of chronic GvHD and can decrease the relapses in patients with high-risk hematological malignancies. It is necessary more follow up and more studies to confirm this preliminary results.

[P115]

		N (28)	%
<b>Median age ( years)</b>		46,5 ( 24-70)	
<b>Gender</b>	Male	16	57.1
	Female	12	42.9
<b>Disease</b>	AML	12	42.9
	ALL	1	3.6
	MDS	2	7.1
	HL	8	28.6
	NHL	2	7.1
	Other	3	10.7
	<b>Disease status at SCT</b>	CR1	14
	Further CR	4	14.3
	PR	8	28.6
	Active disease	2	7.1
<b>DRI refinement</b>	Low	6	21.4
	Intermediate	15	53.6
	High	7	25
<b>HCT-CI score</b>	0-1	3	10.7
	2-3	14	1
	>3	11	50
			39.3
<b>Conditioning regimen</b>	Non myeloablative	20	71.4
	Myeloablative	8	28.6
<b>Stem cell source</b>	Bone marrow	24	85.7
	Peripheral blood	4	14.3
<b>Total number of cell infused (Bone Marrow).</b>	CD34+ cells (x10e6/kg)	2.2 (0.98-5.02)	
	CD3+cells (x10e7/kg)	3.25 ( 2.1-7.5)	
<b>Total number of cell infused (Peripheral Blood).</b>	CD34+ cells (x10e6/kg)	4.36 ( 3.84-4.50)	
	CD3+cells (x10e7/kg)	3.50 (2.07-4.24)	

**Disclosure of conflict of interest:** None.

## Haplo data

### P116

#### Myeloablative T-cell replete peripheral blood stem cell (PBSC) haploidentical (Haplo) transplant with and without pre-transplant steroid with intermediate and high risk hematological malignancies

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Myeloablative T-cell Replete Peripheral Blood Stem Cell (PBSC) Haploidentical (Haplo) Transplant with and without Pre-transplant Steroid with Intermediate and High Risk Hematological Malignancies. T-cell replete haplo 'bone marrow' transplant with post-transplant cyclophosphamide (PTCy) yields promising outcome. Corticosteroids are avoided until administration of PTCy to maximize clearance of alloreactive T cells. We report the use of T-cell replete PBSC haplo transplant with myeloablative regimens with and without pre-transplant high-dose steroid premedication. This is a retrospective analysis of 29 patients who received haplo PBSC transplant (between October 2012 and March 2016) after MA regimens. Myeloid malignancy ( $n=20$  except 1 patient with SAA) received fludarabine (FLU)/busulfan  $\pm$  TBI 4Gy, while lymphoid malignancies ( $n=9$ ) received FLU/TBI 8Gy or CY/TBI 12Gy. All patients received G-CSF-mobilized T-cell replete PBSC from a haplo donor. GVHD prophylaxis was PTCy 50 mg/kg on day +3/+4, tacrolimus (D+5 to +180), and mycophenolate (D+5 to 35). The median duration of follow up of surviving patients is 21 months. Median age was 45 (17–66) years, 17 patients (59%) were male, 14 (48%) were African American, and 14 patients (48%) had comorbidity index (HCT-CI)  $\geq 3$ . All patients had hematological malignancy (except 1 patient with SAA) including 12 patients (43%) not in CR. Disease Risk Index was high/very high in 12 (42%) and intermediate in 14 (48%). On the day of transplant, 14 patients (48%) did not receive steroid premedication (=NO-steroid group), while 15 patients (52%) received 125 mg of methylprednisolone 30 minutes prior to PBSC product infusion (=YES-steroid group). All the following outcomes are described in the 'NO-steroid' vs 'YES-steroid' group, respectively. Cumulative rate (CI) of ANC engraftment ( $\geq 500/\mu\text{L}$ ) on day +28 was 79% (95% CI 60–100%) and 93% (95% CI 81–100) ( $P=0.07$ ). CI of platelet engraftment ( $\geq 20\,000/\mu\text{L}$ ) on day +56 was 71% (95% CI 51–99%) and 90% (95% CI 74–100%) ( $P=0.2$ ). Primary engraftment failure was observed in 3 patients; 1 in YES-steroid and 2 in NO-steroid. No primary engraftment failure was observed with myeloablative TBI (8–12 Gy) ( $n=9$ ). CI of aGVHD GII-IV (day+100) was 21% (95% CI: 8–58%) and 28% (95% CI: 12–64%) ( $P=0.77$ ). CI cGVHD (1 year) was 42% (95% CI 20–90%) and 49% (95% CI 27–87%) ( $P=0.48$ ). CI of relapse (1 year) was 22% (95% CI 7–75%) and 7% (95% CI 1–47%) ( $P=0.71$ ). CI of non-relapse mortality (NRM) (1 year) was 51% (95% CI 29–90%) and 38% (95% CI 19–77%) ( $P=0.48$ ). Post-infusion non-infectious fever (D0 to +3) was observed in 12/14 (86%) and 12/15 patients (80%). Median Tmax was 103F and 102F ( $P=0.38$ ). Only 1 patient in the NO-steroid group developed life-threatening cytokine release syndrome and survived. No difference of viral reactivation was noted between groups. CMV reactivation occurred in 7 (50%) and 13 patients (87%), BK reactivation in 20% (in both groups), HHV6 in 36% and 40%, EBV in 14% and 7%. The 18-month overall survival was 46% (95% CI 17–74%) and 60% (95% CI 35–85%) ( $P=0.53$ ). The 18-month disease-free survival was 41% (95% CI 15–68%) and 53% (28–79%) ( $P=0.46$ ). T-cell replete haplo PBSC transplant is effective therapy for patients with high-risk hematological

malignancies. High-dose steroid premedication with PBSC infusion neither influences transplant outcome nor prevents post-infusion febrile reaction.

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### P117

#### Intrabone transplant of unwashed CB in hematological malignancies: engraftment and safety

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Cord blood transplant (CBT) in adult patients (pts) is limited by the risk of graft failure or delayed engraftment due to low cell counts. To improve the capacity and speed to engraft, intrabone (IB) CBT technique has been investigated, showing high rate of engraftment and low acute GVHD, also when compared with double CB transplant. CB Units washing procedure has been suggested to remove DMSO toxic potential effect. We report our experience in 15 adult pts with hematological malignancies receiving IB unwashed CB in an attempt to reduce the loss of progenitor cells and the risks associated with cell-washing procedure. Between 2009 and 2016 we performed 15 allogeneic hematopoietic stem cell transplant (HSCT) using unwashed CBU as a source and infusing them IB. All pts were adult and suffering from hematological malignancies. This population was characterized by very high-risk and advanced phase disease. All pts received a CB HSCT because of unavailability of sibling or matched unrelated donors. Eleven pts received a Treosulfan-based myeloablative conditioning regimen and a Sirolimus-based GHVD prophylaxis; four pts received Busulfan-based myeloablative conditioning and a Cyclosporine-based GHVD prophylaxis. CB units were thawed and diluted with albumin-dextran solution immediately before the transplant. This 'no-wash' dilution was implemented to reduce product manipulation that may results in cell loss. Furthermore, graft manipulation risks potential contamination, requires increased technologist time, and delays time to infusion. The IB infusion was performed under local anesthesia and with short conscious sedation, at bedside in the BMT ward. The infusion was performed monilaterally or bilaterally according to the volume to be infused. Starting from a 10% DMSO concentration in the CB units before the dilution, the graft products contained a median of 3.6% DMSO (range: 2.3–6.9) at IB-HSCT. The median CD34+ cells infused were  $0.09 \times 10^6/\text{kg}$  b.w. (range: 0.03–0.82). The median mono-nucleated cells were  $15.66 \times 10^6/\text{kg}$  b.w. (range: 4.7–36.8). The median CD3+ T-cells were  $2.6 \times 10^6/\text{kg}$  b.w. (range: 0–5.54). The median infused volume was 75 mL (range: 60–215). No procedure-related adverse events were observed, nor related to the IB technique, neither to the sedation. Of the 15 transplanted pts, 9 were evaluable for engraftment (1 patient rejected the graft and 5 patients died before day +30 because of severe infections). All 9 achieved ANC  $> 0.5 \times 10^9/\text{L}$  after a median of 24 days (range: 16–45) and 8 achieved PLT  $> 20 \times 10^9/\text{L}$  at a median of 45 days (range: 25–141). Three patients developed grade III–IV acute GVHD grade. According to extreme heterogeneity of the population no correlations with relapse incidence and disease-free survival could be evaluated. IB infusion of unwashed CB is feasible, safe, easy to perform. No adverse events related to the procedure were documented. No DMSO toxicity was documented. Engraftment was obtained in all evaluable pts. Our data confirm that direct IB CBT overcomes the problem of

graft failure even when low numbers of CB cells were transplanted, thus leading to the possibility of using of this technique in a large number of adult pts, for whom this approach represents the sole possibility of long-term survival. The 'no wash' CB dilution can also help the implementation of IB transplant thanks to the easier graft manipulation.

[P117]

Patients, disease and transplant characteristics

Male/Female	8/7
Median Age (range)	43 (22-63)
Weight (range)	70 (50-85)
Disease:	
- ALL	4
- NHL	2
- AML/MDS	9
Advanced phase (relapse or CR>2)	11
Active Disease	7
Previous Allo-Transplant	10
HLA matching:	
- 4/6	6
- 5/6	7
- 6/6	2

**Disclosure of conflict of interest:** None.

**P118**

**Lower incidence of cGVHD after cord blood transplantation for hematological malignancies in comparison with hematopoietic stem cell transplantation from other donors: 20 years' experience in a single institute**

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The outcome of cord blood transplantation (CBT) for hematologic malignancy was investigated. However the incidence of GVHD is not accurately known. The goal of this study was to compare the outcome of CBT with allogeneic hematopoietic stem cell transplantation (allo-HSCT) from other sources, mainly unrelated bone marrow (URBM). Patients' characteristics: 755 patients who underwent allo-HSCT, between 1990 and 2015 in our hospital were retrospectively analyzed. Donor sources were cord blood cell ( $n=112$ ), URBM ( $n=372$ ), HLA matched sibling bone marrow (Sibling BM) ( $n=166$ ), and HLA matched sibling peripheral blood stem cell (Sibling PBSC) ( $n=105$ ). In CBT, the median age was 38.5 (17-68), and the diagnosis included AML (69), ALL (15), MDS (13), CML (4) and other (11). The disease risk was good in 58 and poor in 54. Disease risk was slightly higher in comparison with other sources. Prophylaxis of acute GVHD was tacrolimus, short-term methotrexate (88), cyclosporine, short-term methotrexate (22) and others (2). The 5-year overall survival (OS) rate after CBT (1990-2004) and CBT (2005-2015) was 15.8% and 49.1%, respectively, and therefore the OS and rate of CBT improved over periods and became comparable with URBM (2005-2015) (49.2%). The 5-year relapse rate after CBT (2005-2015) and URBM (2005-2015) was 32.3% and 32.1%, respectively. The 5-year non-relapse mortality (NRM) after CBT (1990-2004)/CBT (2005-2015) /URBM (1990-2004)/URBM (2005-2015) was 62.6%/39.5%/48.7%/30.3%, respectively. Cumulative incidence of acute/chronic GVHD was 13.4%/5.0% in CBT, but 26.0%/32.1% in URBM. The causes of death within 100 days after CBT were TRM 83.3% (infection 50.5%, thrombotic microangiopathy (TMA) 13.3%, organ failure

10.0%, engraftment failure 6.7%, acute GVHD 3.3%), relapse 10.0% and other 6.7%. In CBT cases, engraftment failure after allo-SCT was observed in 24 cases (21.4%) which is higher than that among URBM (8.6%), 32 out of 372. 10 CBT cases underwent the second allo-HSCT and 9 patients achieved engraftment and 7 patients were alive at 100 days after allo-HSCT. 6 of them survived at 2 years after allo-HSCT. Our results suggest that the outcome of CBT has improved in recent years. Moreover, CBT has an advantage in the least cumulative incidence of acute/chronic GVHD. CBT may well create the best outcome in the future.

**Disclosure of conflict of interest:** None.

**P119**

**Patients with chronic active Epstein-Barr virus infection Outcome of umbilical cord blood transplantation in adult patients with chronic active Epstein-Barr virus infection**

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Chronic active Epstein-Barr Virus (EBV) infection is a major type of EBV-associated T/NK-cell lymphoproliferative disorders (LPD) in childhood. However, young adults rarely develop chronic active EBV infection (CAEBV), and shows more aggressive features than that of childhood. Umbilical cord blood transplant (UCBT) is a possible treatment option for CAEBV patients who have no HLA-matched donor, but there is little information available about the efficacy and safety of UCBT for adult patient with CAEBV. We analyzed six adult patients with CAEBV who underwent a single-unit UCBT between 2010 and 2016 at our institute (including a case reported in 2014[1]). The diagnosis of CAEBV was made according to the criteria proposed in 2005 [2]; persistent infectious mononucleosis (IM)-like symptom and detection of increased EBV genomes in peripheral blood mononuclear cells (PBMC). EBV-DNA load was measured using real-time quantitative PCR. Median patient age at diagnosis was 27 (23-39) years. Target cells of EBV-infection were CD4+T cells ( $n=4$ ) or NK cells ( $n=2$ ). Median EBV-DNA load was  $2.6 \times 10^4$  copies per microgram of DNA in PBMC (range:  $1.6 \times 10^3$ - $1.3 \times 10^5$ ) at the diagnosis. All patients were given prednisolone and cyclosporine, and then etoposide ( $n=2$ ) or combination chemotherapy ( $n=4$ ) before transplant. EBV load has slightly decreased to a median of  $1.3 \times 10^4$  copies (range:  $4.0 \times 10^2$ - $7.1 \times 10^4$ ), but disease status was active at UCBT in all. Median time from the diagnosis to UCBT was 101 days (range: 62-440). One patient received total body irradiation (TBI) 12Gy + cyclophosphamide (CY) 120 mg/kg + cytosine arabinoside 8 g/m<sup>2</sup>, and the other five patients received fludarabine (Flu) + melphalan (LPAM) 80-140 mg/m<sup>2</sup> or CY 120 mg/kg with TBI 4Gy before UCBT. Umbilical cord blood (UCB) was 4/6 HLA-matched to the recipients. Median number of infused UCB CD34+ cells was  $0.87 \times 10^5$ /kg (range:  $0.76$ - $1.93 \times 10^5$ ). GVHD prophylaxis was consisted of tacrolimus + methotrexate or mycophenolate mofetil. Neutrophil engraftment and complete donor chimerism were achieved in four patients, but two of them developed secondary graft failure (GF) early after engraftment. The other two patients developed primary GF. Second UCBT was successfully performed in the 4 patients with GF a median of 31.5 days (range: 28-58) after the first UCBT. EBV genomes in PBMC became undetectable immediately after UCBT. At a median follow-up of 873 days (range: 54-2446), EBV-DNA was undetectable or very low, and IM-like symptoms were resolved in all cases. However, at 7-9 months after UCBT, two patients developed EBV+ B-cell LPD derived from donor cells, that was successfully treated with rituximab therapy. This study suggested that UCBT could eradicate EBV-infected CD4+ T cell- or NK cell- clones. UCBT can be a treatment option for adults with CAEBV. Rituximab monotherapy was effective for post-transplant LPD from donor B cells. However, a high incidence of GF was observed in patients receiving reduced-

intensity conditioning of Flu/LPAM or CY /low-dose TBI. Further studies are needed to find more optimal regimens for stable engraftment of UCB in adult patients with CAEBV.

#### References

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**Disclosure of conflict of interest:** None.

#### P120

##### **ABO incompatibility in allogeneic hematopoietic stem cell transplantation: 15-years experience of R.M.Gorbacheva memorial institute of children oncology, hematology and transplantation**

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There is an increased incidence of ABO incompatibility—50–60%, in allogeneic hematopoietic stem cell transplantation (alloHSCT) in patients who are Russian citizens as a result of the variability of genetic polymorphism in the multi-ethnic population and a significant number of unrelated donors from international bone marrow registries. ABO incompatibility in different types of alloHSCT may be an additional aggravating factor for the development of immunological complications and decrease effectiveness of treatment, but the data is still controversial [1]. From May 1999 to December 2015 in Raisa Gorbacheva Memorial Institute for Children Oncology, Hematology and Transplantation 1131 patients with leukemia, malignancies and hereditary diseases were included to the study, who were performed 1428 HSCT: allogeneic unrelated—814 (57%); allogeneic related—344 (24.1%); haploidentical—267 (18.7%); umbilical cord blood in 3 patients (0.2%). Age was 0–76, median—25 years. Patients were predominantly with acute myeloid leukemia—37% ( $n=602$ ), acute lymphoblastic leukemia—30% ( $n=501$ ) and chronic myeloid leukemia—6% ( $n=94$ ). **Results:** In 54.6% of cases ( $n=780$ ) ABO incompatibility was determined: major—37.8% ( $n=295$ ); minor—45.4% ( $n=354$ ); combined—16.8% ( $n=131$ ). ABO incompatibility in alloHSCT did not influence overall survival ( $P=0.56$ ) and frequency of acute graft versus host disease (GvHD) ( $P=0.2$ ). Also there was no difference in overall survival depending on combination of condition regimen and ABO incompatibility: reduced intensity (RIC) or myeloablative (MAC) ( $P=0.7$ ). An increased frequency of acute GvHD was observed in RIC and ABO incompatibility (30.8%) compared to MAC (15.3%,  $P=0.002$ ). ABO incompatibility was not a major factor (log worth 0.87) which influenced the fact and speed of donor's transplant engraftment in comparison to level of HLA-compatibility (15.1), hematopoietic stem cell source (7.05) and type of HSCT. But the presence of major ABO incompatibility increase the period of erythroid recovery ( $P=0.01$ ) as reflected in the higher amount of blood transfusions. Complications caused by ABO incompatibility were identified in 2.4% of all cases ( $n=34$ ) including acute and delayed hemolysis, partial red cell aplasia and immune thrombocytopenia. Conclusion. The presence of ABO incompatibility is not a limiting factor to perform alloHSCT, however, it demands high quality prophylaxis and accurate transfusion therapy depending on ABO incompatibility type to prevent immune complications.

**Keywords:** Allogeneic HSCT, ABO-incompatibility.

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**Disclosure of conflict of interest:** None.

## Stem cell donor

#### P121

##### **Previously published**

#### P122

##### **Circulating CD34+ cell numbers after re-mobilization of related donors in view of second allo-HSCT or immunoselected allogeneic CD34+ cell infusion ('boost') are lower to those observed at first mobilization: A single institution retrospective paired analysis**

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Poor graft function or graft failure have become common indications for infusion of immune-selected CD34<sup>+</sup> cells ('boost') or second unprocessed allo-HSCT, creating the need for re-mobilization of the same related or unrelated. We retrospectively compared the results of two consecutive cycles of rh-G-CSF treatment and peripheral blood progenitor cell collections in 20 related donors cared for at our Institution between 2008 and 2016. Mobilization consisted of the administration of rh-G-CSF at a dose of 10 µg/kg per day injected in the evening, and apheresis was started in the morning of the fifth day after the fourth dose of rh-G-CSF. Collection was performed with a SPECTRA or SPECTRA OPTIA cell separator (Terumo BCT).

Eleven out of 20 were haplo-mismatched donors and 9 were HLA matched donors. Four donors were re-collected because of recipient graft failure and 16 because of poor graft function; in the latter situation, immunomagnetic selection of CD34<sup>+</sup> cells was performed on the collected cell product prior to infusion into the recipient, using the CliniMACS medical device, as previously published. Median donor age was 43 years (range: 18–66) at time of first donation, median weight 70kg (45–112) and BMI 24 (18–34). Median delay between mobilizations 1 and 2 was 192 days (28–1530). Interestingly, the median delay between collections was 119 days (43–701) in the haplo-mismatched setting and 231 (28–1530) in the matched setting. Median number of circulating CD34<sup>+</sup> cells/µL after the first 4 injections of rhG-CSF was 61 vs 37 at the first and second mobilization cycles ( $P < 0.001$ , Table 1). Seven out of 20 donors (35%) requested more than one apheresis session to obtain the target number of collected CD34<sup>+</sup> cells during the first cycle, as compared to 12 out of 20 (55%) for the second cycle: this is largely due to the higher target of CD34<sup>+</sup> cells for the second collection, expecting that the median CD34 recovery after immunomagnetic selection is 60% in our experience. Our study shows that a second cycle of mobilized peripheral blood progenitor cell collection from related donors is associated with a significant reduction in response to hematopoietic growth factors and mobilization capacity. This information allows planning the number of aphereses at the second cycle—and subsequently the number of immunoselection procedures to be carried out—taking into account the higher CD34<sup>+</sup> cell dose target needed for subsequent immunomagnetic selection.

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	first mobilization	second mobilization
median dose of rh-G-CSF (µg/kg)	10,0 (8 - 13)	10,2 (8 - 14)
median circulating CD34+ /µl at day 5	61 (13 - 182)	37 (12 - 117)
median delta circulating CD34+ /µl (first - second mobilization)	12 (-5 - 92)	
% of donors requesting more than one apheresis	35%	55%

Disclosure of conflict of interest: None.

P123

**CMV seropositive donor selection for CMV seropositive stem cell transplant recipients reduces CMV reactivation frequency and duration**

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CMV reactivation remains one of the main complications after allogeneic stem cell transplantation (HSCT), requiring antiviral therapy, causing myelosuppression, prolonged hospitalization, higher treatment costs and mortality. CMV seronegative donors are recommended for CMV seronegative recipients. However data about donor selection for CMV positive (CMV-pos) recipients is not conclusive. Some studies showed that selecting CMV-pos donors for CMV-pos patients might be beneficial. CMV-seropositivity is very high in Lithuania among healthy blood and bone marrow donors (65%) and even higher among HSCT recipients (up to 90%), so donor selection for CMV-pos recipients is an object of interest. Retrospective analysis of CMV reactivations in CMV-pos allogeneic HSCT recipients (transplanted during 2005–2014 year in Vilnius University Hospital) who survived at least 6 months post HSCT was performed. Data about CMV reactivation frequency, time post HSCT, duration, maximal CMV DNA copy number at each reactivation collected. CMV reactivation was considered when CMV DNA copies detected >500/mL in patient's blood. Statistical analysis conducted using SAS 9.2; Student's tests

for statistical significance; Kaplan–Meier methods for overall survival. Among 316 allogeneic HSCT recipients 286 (90.5%) were CMV-pos. 286 CMV-pos allo-HSCT recipients were further analysed. 150 of them received graft from CMV-pos (POS/POS group) and 136—from CMV-seronegative donors (POS/NEG group) and 136—patients in POS/NEG group experienced CMV reactivation in first 6 months post HSCT in comparison to POS/POS group (83.1% vs 65.3%,  $P < 0.05$ ). POS/NEG group patients had more CMV reactivations (2.5 vs 2 times in 6 months post transplant period,  $P < 0.05$ ), reactivations were diagnosed earlier post transplant (50.7 vs 123 days post HSCT,  $P < 0.0001$ ), had longer duration (60.9 vs 40.3 days,  $P < 0.0001$ ) and larger maximal CMV DNA copy number (285107,9 vs 24339.4 copy/mL,  $P < 0.0001$ ) in comparison to POS/POS group patients. POS/POS group patients showed tendency for better survival than POS/NEG group patients, however did not reach statistical significance. In a univariate analysis only HLA mismatch and donor CMV seronegativity were factors statistically significantly associated with CMV reactivation. Donor CMV serostatus is significant factor selecting donor for allo-HSCT recipients. According to our findings, selecting CMV-pos donor for CMV-pos recipient may reduce CMV reactivation frequency and duration.

Disclosure of conflict of interest: None.

P124

**Comparable outcomes using a CMV-matched or a mismatched donor for CMV+ patients undergoing T-replete haplo-HSCT with PT-Cy for acute leukemia: A study from IDWP and ALWP of the EBMT**

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Figure 1a: NRM in the CMV-matched and mismatched group

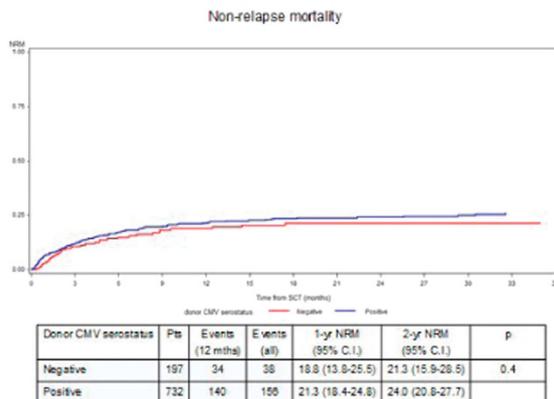
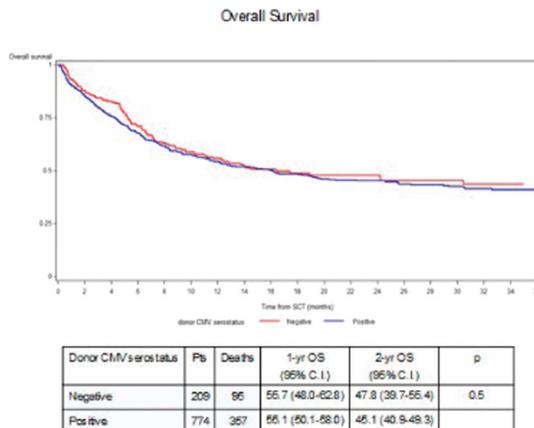


Figure 1b: OS in the CMV-matched and mismatched group



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CMV status is a major determinant of transplant outcome (1–3), but its role in the setting of haploidentical T-replete HSCT with post-transplant cyclophosphamide (PT-Cy) has been little studied. Since usually the potential haploidentical donors are multiple, a better definition of donor CMV serostatus may help to improve the search for the optimal haplo-donor. A recent analysis (4) failed to demonstrate any significant role of donor CMV serostatus, but it remains unknown if this was real or due to the relatively low numbers in the series (4). A registry study, with larger numbers, is the most suitable setting to address this question. Data on CMV+ patients receiving haplo-HSCT and PT-Cy-based GvHD prophylaxis for acute leukemia from 1 January 2010 to 31 December 2015 have been retrospectively analyzed. CMV serological status of donors, main patients' and donors' characteristics as well those of transplant procedure have been studied and correlation analyses were performed with clinical endpoints. Due to a potential distinct effect of donor CMV serostatus according to the intensity of conditioning (1), analyses of NRM have been stratified separately for RIC and MAC. **Results:** a total of 983 CMV+ patients were identified, with a median follow-up of 1.6 years (95% C.I.: 1.4–1.8). Among these,  $n=774$  (79%) and  $n=209$  (21%) had a CMV+ and CMV-donor, respectively (namely CMV-matched and CMV-mismatched groups). The main significant different characteristics between the two groups were: higher patient's age, lower donor's age, more RIC, and less CR at transplant in the CMV-mismatched group. Scatter plot representing patient age and donor age on the  $n=534$  patient/donor pairs with the information on donor's age available, suggests more direct linear relationship between patient and donor in the CMV-mismatched group, that is, the patient being a parent and the donor being a child. One-year NRM was 21.3% (95% CI: 18.4–24.8) and 18.8% (95% CI: 13.8–25.5) in the CMV-matched and CMV-mismatched groups, respectively ( $P=0.40$ ). NRM was comparable after stratification for the conditioning intensity. OS was similar in both groups, as well as relapse-free survival, relapse incidence, acute and chronic GvHD. Neutrophil engraftment was superior in the CMV-mismatched group: 91.1% vs 85.5%,  $P=0.01$ . Multivariate model confirmed no significant association between donor CMV serological status and NRM, although a trend toward inferior NRM using a CMV-donor is observed (HR 0.69, 95% CI 0.47–1.04,  $P=0.07$ ). **Conclusions:** on a consecutive, registry-based cohort of 983 CMV+ patients with acute leukemia undergoing T-replete haplo-HSCT with PT-Cy, we found similar outcomes using a CMV-matched or a CMV-mismatched donor, thus confirming previous findings. The assessment of the role of donor's age deserves further study in this setting.

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**Disclosure of conflict of interest:** CR is employee of MolMed S.p.A. at the time of submission.

#### P125

##### **Comparison of Mismatched Unrelated Donors and Cord Blood to Matched Unrelated Donors in pediatric patients with acute leukemias or myelodysplastic syndromes in Brazil: A multicenter retrospective study**

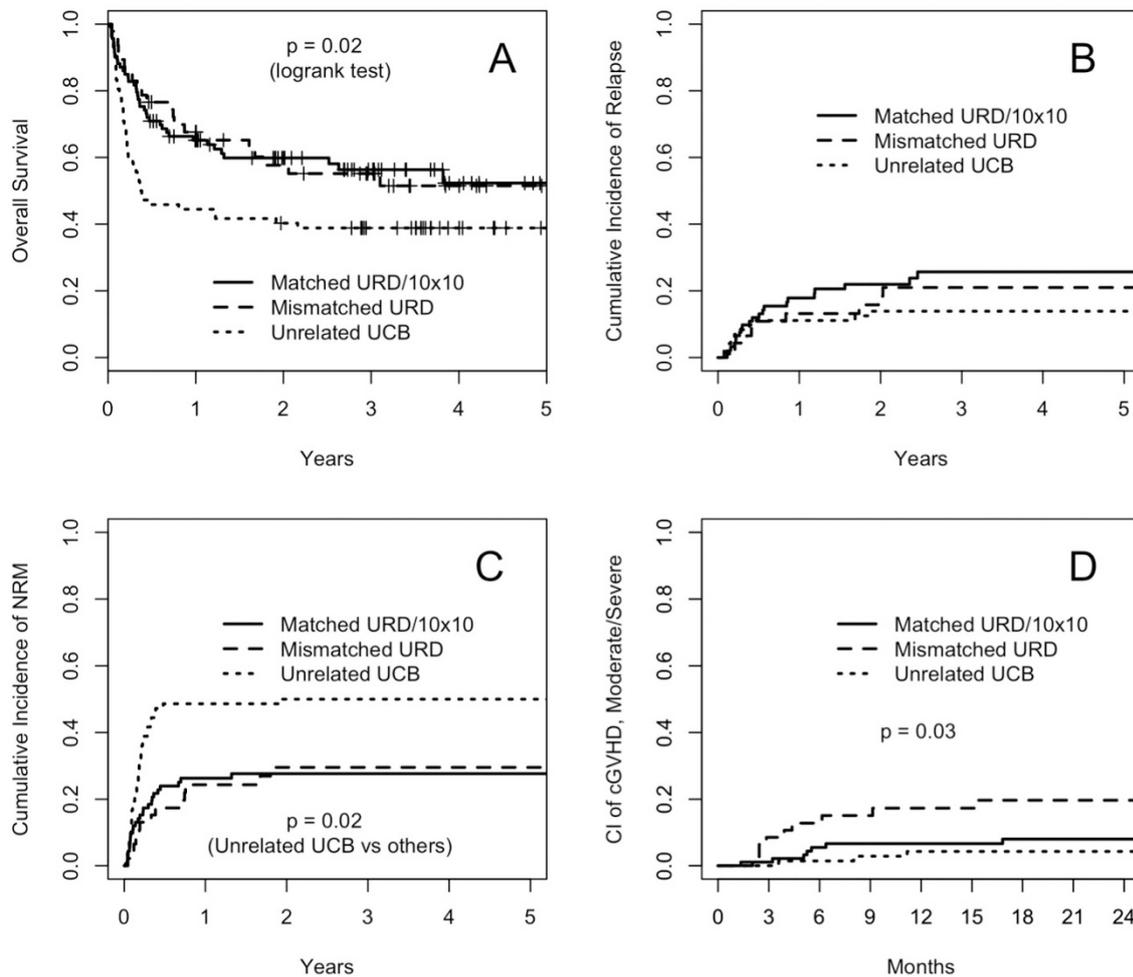
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Selection of the best HSC donor when a matched donor is not available is still a matter of debate, and reports in pediatric population are scarce. This is a retrospective study conducted by Brazilian Society of HSCT (SBTMO), including 11 centers, aimed to compare Matched Unrelated (Matched-URD), Mismatched Unrelated (MM-URD) and Unrelated Cord Blood (UCB) HSCT. ALL or AML/MDS patients < 18 y/o who have received first unrelated HSCT between 2010–2014 were included. HLA 4-digit typing was available for URD; for UCB, HLA class-I 2-digit typing. Overall survival (OS), and cumulative incidence (CI) of aGVHD, cGVHD, NRM and relapse were analyzed. On an unplanned analysis, we fitted a lognormal Bayesian survival model with random effects, imputing the probabilities of UCB matching at 8 loci. A total of 212 patients were included (93 Matched-URD, 47 MM-URD and 72 UCB). Median age was 8.7 y/o. Most patients had ALL (61%). Proportion of early disease in Matched-URD was higher (37%, against 19 and 21%). Matched-URD were 10/10 4-digit HLA matched (except one, for whom DQ was not available), while most of MM-URD (89%) were 9/10 HLA matched. UCB were 6-loci (58%) or 8-loci typed (42%). Based on previous report<sup>1</sup> on extending 6-loci HLA typing to 8-loci UCB, we estimated that 23 UCB were 0–1 mismatched at 8 loci, 26 had 2 mismatches and 23 had 3 or more mismatches. Conditioning regimens were mainly myeloablative, TBI- (59%) or Bu- (33%) based. Grafts were *in vivo* T-cell depleted in 67% of the patients, not balanced between groups ( $P=0.01$ ). With median follow-up of 3.3 years, 3y OS was 56%, 52% and 39% for Matched-URD, MM-URD and UCB, respectively ( $P=0.02$ , log rank test). For Matched-URD, MM-URD and UCB, CI of grades III–IV aGVHD at 6 months were 18%, 13% and 17% ( $P=NS$ ); moderate/severe 3y-cGVHD, 8%, 20% and 4% ( $P < 0.05$ ); 3y-relapse, 26%, 21% and 14% ( $P=NS$ ); and 1y-NRM, 26%, 24% and 49% ( $P=0.06$ ). We found out that primary graft failure occurred in 14 (19%) of UCB, compared to 9% in MM-URD and 3% in Matched-URD. When UCB matching probabilities at 8 loci were imputed and analyzed in a Bayesian model (controlled for age, gender, disease status and diagnosis, and T-cell depletion), survival was inferior in UCB with 3+ mismatches (9.8-times lower median survival,  $_{95}CI$  2.6–39.4), but not with up to 2 mismatches (1.3-time higher median survival,  $_{95}CI$  0.4–3.8). Of note, *in vivo* T-cell depletion marginally impaired survival (2.4-times decrease,  $_{95}CI$  1.0–6.0). **Discussion:** In our population, overall survival achieved with MM-URD was not different to 10/10 Matched-URD, despite higher incidence of moderate/severe cGVHD. On the other hand, survival with UCB was significantly lower. Recent report<sup>2</sup> have shown excellent OS with UCB compared to 8/8 HLA-matched URD. UCB cohort inferior results may have been due to HLA disparity degree, since survival in UCB with up to 2 mismatches (out of 8) was not worse. One limitation of our study is that TNC and CD34 from UCB units were not available, impairing primary graft failure analysis. We have also found that *in vivo* T-cell depletion might have a detrimental effect on survival and should be

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studied further in prospective trials. In conclusion, MM-URD, especially HLA 9/10, is a suitable option when a fully HLA 10/10 Matched-URD is not available. UCB matched at least 6/8 may also be a good option.

**Disclosure of conflict of interest:** None.

**P126**

**Does matched sibling donor transplantation provide better outcome compared to non-sibling matched family donor in children with hematological malignancies?**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a proven treatment for patients with high risk or relapsed hematological malignancy. The probability of having a HLA matched family donor is about 30%. In populations with high consanguinity rates, the probability of having non-sibling matched family donor(MFD) is much higher. To explore the impact of MSD vs non-sibling MFD on outcome of HSCT recipients, we undertook a single center retrospective analysis of pediatric patients transplanted with the diagnosis of hematological malignancy at our center in the last five years.

A retrospective cohort from 2011 to current included 113 pediatric patients with hematological malignancies transplanted from family donors, of which 98 were from MSD and 15 from non-sibling MFD. HLA matched family donors were identified by high resolution allelic typing and were matched 10 of 10 HLA loci. Diseases were ALL ( $n=58$ ), AML ( $n=37$ ), MDS ( $n=7$ ), JMML ( $n=5$ ), KML ( $n=2$ ), NHL ( $n=1$ ) and Hodgkin's disease ( $n=3$ ). Conditioning regimens were TBI or Busulphan-based myeloablative in all patients. The median age of the patients was 9.4 years (range: 5 month–18.9 years). Although peripheral stem cell seemed to be used more commonly in non-sibling MFD recipients (64% vs 42%), the difference was not statistically significant. The median follow up time for alive patients 24 months (1–67 months). Two year overall survival and leukemia free survival did not differ between patients with transplantations from MSD or non-sibling MFD ( $67\% \pm 5.0$  vs  $60 \pm 5.1$ ,  $P=0.31$ ) Similarly, leukemia free survival was not different between MSD and non-sibling MFD transplants ( $58.2\% \pm 13.1$  vs  $58.1\% \pm 13.2$ , respectively). The incidences of Grade II–IV acute GVHD in MSD and non-sibling MFD transplants were % 18 and %13, respectively. The incidences of relapses were 28% in MSD transplants and 20% in MFD transplants and the difference was not significant ( $P=0.49$ ). These data show that the results of HSCT from non-sibling MFD is comparable to HSCT of MSD in children with hematological malignancy. Our data emphasize the need for extended high resolution family typing for patients in regions where there is high rate of consanguinity.

**Disclosure of conflict of interest:** None.

## P127

### Donor-recipient RH incompatibility is a risk factor for mortality after pediatric matched related allogeneic hematopoietic stem cell transplantation

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Optimal donor selection is critical to achieve the best outcome after allogeneic hematopoietic stem cell transplantation (allo-HSCT). There is no consensus regarding the effect of donor-recipient RH incompatibility on survival after matched-related donor (MRD) allo-HSCT in children and adolescents. This abstract aims to study this effect in a single-institution cohort over a period of 11 years. This is a retrospective chart review for all patients aged < 21 years who underwent allo-HSCT at the American University of Beirut Medical Center between August 2005 and June 2016. A total of 70 patients with a median age of 11 years (range: 0.6–21 years) underwent allo-HSCT from MRD for the following diseases: leukemia ( $N=36$ ), bone marrow failure ( $N=15$ ), thalassemia ( $N=8$ ), SCID ( $N=5$ ), metabolic diseases ( $N=4$ ) and lymphoma ( $N=2$ ). The stem cell source was bone marrow for 56 patients (80%) and mobilized peripheral blood stem cells for 14 patients (20%). The grafts contained a median of  $5.3 \times 10^9/\text{kg}$  total CD34 cells. TBI was used in 13 patients (18%). All but 4 patients achieved sustained neutrophil and platelet engraftment. After a median follow-up of 47 months (range: 4–135 months), the 2-year overall survival rate was 70% (95% CI: 57–79%). By multivariate analysis using cox proportional hazard regression model looking at the following factors for overall mortality: diagnosis, recipient's age, donor's age, the use of TBI, stem cell source, CD34 count, donor-recipient ABO incompatibility, donor-recipient RH incompatibility, and donor-recipient sex-mismatch, the only statistically significant risk factor for mortality was donor-recipient RH incompatibility (HR: 3.26,  $P=0.04$ ). This risk was not statistically significant when looking at transplant-related mortality (HR: 3.9,  $P=0.17$ ) and relapse-related mortality for malignant diseases (HR: 3,  $P=0.12$ ). There was no association between the incidence of acute or chronic GvHD and RH incompatibility. Donor-recipient RH incompatibility was associated with an increased risk of mortality in children and adolescents undergoing MRD allo-HSCT. Further studies with larger number of patients are needed to confirm this finding.

**Disclosure of conflict of interest:** None.

## P128

### Effect of iron or Vitamin B12 deficiencies on *in vitro* colony forming capacity of peripheral blood-derived hematopoietic stem cells in children

NY Özbek, MM Zabun, Y Köksal and M Özgüner

Iron deficiency (ID), ID anemia (IDA) and vitamin B12 deficiency (Vit-B12D) are common disorders in developing countries. In urgent situations, children with these disorders could be donors before treatment. In this study, we investigated capacity of peripheral blood-derived hematopoietic stem cells to develop colony-forming units (CFU) in children with ID and vit-B12, *in vitro*. **Patients and Methods:** We included 102 children (age 6 months–18 years) in the study in 5 groups: children with ID ( $n=15$ ); children with IDA ( $n=20$ ); children with vit-B12D ( $n=12$ ); children with both ID and vit-B12D (I/Vit-B12D;  $n=18$ ); and control children ( $n=37$ ) who has normal peripheral blood findings, and normal ferritin and vit-B12 levels. From each child complete blood counts (CBC), and levels of ferritin, vit-B12, and C-reactive protein (CRP) have been obtained. WHO criteria, adjusted for age and sex, have been used for definition of anemia, ID, IDA and vit-B12D. Four mL peripheral blood drawn into tubes with EDTA has been used for CFU analysis. Mononuclear cell suspension

( $1.5 \times 10^6$  cell/mL), obtained from peripheral blood by Ficoll-Hypaque density gradient separation method, has been cultured in dishes containing semi-solid agar culture medium (Methocult, H4434 Classic, Stem Cell Technologies, Canada) in appropriate conditions. After 2 weeks, number of CFU colonies [burst forming erythroid (BFU-E); colony forming unit-granulocyte macrophage (CFU-GM); colony forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte (CFU-GEMM)] have been investigated by an inverted microscope. **Results:** Statistical analysis showed no difference between groups for age, sex, CRP levels, and CFU-E, CFU-GM and CFU-GEMM numbers. However, expected differences between groups were present concerning mean values of hemoglobin, ferritin and vit-B12 levels, mean corpuscular volume (MCV), and red cell distribution width (RDW) (Table 1). Discussion: This study shows *in vitro* proliferation capacity of peripheral stem cells has not been influenced by ID, IDA, vit-B12D, or I/Vit-B12D. Our results may indicate normal grafting ability of peripheral stem cells obtained from donors with iron, vit-B12 or I/Vit-B12 deficiencies for hematopoietic stem cell transplantation. However, *in vivo* analysis should also be performed in order to reach a definite conclusion.

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Table 1: Data of children in the study [mean (Standard Deviation)]

Parameter	ID (n=15)	IDA (n=20)	Vit-B12D (n=12)	I/Vit-B12D (n=18)	Control (n=37)	P
Hb (g/dL)	12.5 (0.8)	9.5 (1.3)	12.7 (1.2)	10.8 (2.3)	13.3 (1.2)	<0.001
MCV (fL)	76.3 (5.9)	62.7 (8.0)	77.4 (4.5)	70.7 (8.3)	79.5 (5.2)	<0.001
RDW (%)	14.8 (1.0)	19.2 (4.5)	15.9 (5.0)	17.5 (4.5)	14.8 (2.9)	<0.001
Ferritin (mcg/L)	8.8 (2.2)	4.8 (3.1)	27.5 (18.6)	6.5 (3.5)	41.4 (46.9)	<0.001
Vit-B12 (pMol/L)	384 (140.9)	458 (188)	172.7 (33.2)	149.9 (36.9)	378.5 (175)	<0.001
CRP (mg/dL)	0.2 (0.1)	0.2 (0.2)	0.1 (0)	0.3 (0.3)	0.3 (0.3)	0.068
BFU-E number	43.2 (23.3)	39.2 (25.7)	48.7 (22.8)	45.4 (17.2)	37.6 (24.9)	0.578
CFU-GM number	32.6 (16.7)	25.3 (12.1)	37.1 (13.2)	33.6 (10.7)	29.1 (19.3)	0.248
CFU-GEMM number	7.9 (4.5)	7.2 (4.1)	9.3 (2.9)	9.0 (3.6)	6.6 (4.4)	0.160

**Disclosure of conflict of interest:** None.

## P129

### Efficiency of day 4 compared to day 6 stem cell mobilization in allogeneic donors

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Granulocyte colony stimulating factor (G-CSF) given for 4–6 days is commonly used for mobilization of allogeneic stem cell donors. The optimal days of G-CSF administration is still debatable. The primary objective of this study is to compare the yield of stem cell mobilization, assessed using CD34+ cell count, between Day 4 and Day 6. Secondary objectives include the assessment of the impact of donor's age, weight, mean corpuscular volume and blood group on the difference in the CD34+ cell count. In this retrospective study we included all allogeneic stem cell donors mobilized with G-CSF for 6 days from January 2003 till October 2015 in the bone marrow transplantation unit at Sultan Qaboos University Hospital. Of 106 donor records reviewed, 84 were with available data and selected for the study. Descriptive and analytical statistics were performed using STATA 13.1. We included 84 donors with median age and weight of 19 years and 60 kg, respectively. The median Day 4 WBC and CD34+ cell count were  $37.4 \times 10^9/\text{L}$  and  $54 \times 10^6/\text{L}$  respectively; while the median Day 6 WBC and CD34+ cell count were  $44.4 \times 10^9/\text{L}$  and  $86 \times 10^6/\text{L}$ , respectively, (Figure) with a statistically significant difference from Day 4 ( $P < 0.001$ ). In the

multivariable model, there were no significant impact of donor's age ( $P=0.215$ ), weight ( $P=0.108$ ), height ( $P=0.428$ ) and mean corpuscular volume ( $P=0.263$ ) on the difference in CD34+ cell yield. However, donor's blood group AB predated a significantly higher difference ( $P=0.036$ ). Six days of G-CSF mobilization achieves higher CD34+ cell count than 4 days in allogeneic stem cell donors especially in donors with blood group AB. However, CD34+ cell count on Day 4 is high enough to allow for successful mobilization. Appropriately designed prospective trial is needed to confirm these results.

**Disclosure of conflict of interest:** None.

### P130

#### Ethnicity is an important determinant of stem cell donation decisions in unrelated donors

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There are known differences between individuals on an unrelated HSC donor register who decide to proceed with verification typing (VT) vs those who choose not to. In the Anthony Nolan registry, white British donors are more than twice as likely as other ethnic groups to continue with testing at VT (OR 2.44;  $P < 0.001$  (unpublished data)). The purpose of this study was to explore differences in key characteristics between white British donors and British donors from other ethnic groups with a view to developing interventions to reduce VT stage attrition. Study recruitment occurred April 2013–May 2016. All donors not proceeding at VT were invited to participate, and a stratified random sample of those proceeding at VT were recruited to meet pre-determined targets for each ethnic group. Data were collected via structured interview (telephone or online). 4 broad categories of participant characteristics were assessed: demographic, culturally related, psychosocial, and donation-related. Measures were previously validated scales with established

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Donor characteristic	WB	NWB	P
Completed higher education	59%	77%	<0.001
Blood or platelet donor	32%	23%	0.02
Registered organ donor	45%	21%	<0.001
Religious service attendance/month (mean)	0.63	4.93	0.001
Importance of religion (range 1–9)	2.96	5.58	<0.001
Religious objections (range 1–5)	1.70	1.79	0.166
Family loyalty (range 1–9)	2.66	2.84	0.003
Medical system mistrust (range 1–5)	2.42	2.61	0.001
HSC allocation mistrust (range 1–5)	1.78	2.04	<0.001
Felt well informed at joining register (range 1–4)	2.79	2.55	0.008
Feel well informed now (range 1–4)	3.33	3.16	0.012
Remember joining	58%	49%	0.009
Remember donation methods	34%	22%	0.02

psychometric properties either created for, or used in other donation-related settings. For analyses donors were divided into two groups based on ethnicity: white British (WB), and non-white British (NWB). **Results:** 170 WB donors and 187 NWB donors completed interviews Donors proceeding at VT were more likely than their counterparts to participate in the study (66% vs 25%,  $P < 0.001$ ). Mean donor age was 33.8 with no difference between ethnic groups and 43% of donors in both groups were female. NWB were statistically more likely to have completed higher education, and have a stronger religious affiliation. In contrast they were less likely to be blood or organ donors. NWB also described greater mistrust of the medical system and of HSC allocation.

NWB donors were more likely to have joined the register at a recruitment event ( $P=0.012$ ) or a place of worship ( $P=0.012$ ), while WB donors were more likely to have joined online ( $P=0.013$ ). WB donors reported significantly higher scores regarding feeling well informed about donation both at the point of joining, and at the point of VT and were more likely to remember joining the register and the two donation methods. This study highlights important differences in demographics, culturally related variables and donor interaction with the register between white British donors and donors from other ethnic backgrounds. Given the higher rate of VT attrition in NWB donors, these findings could be used to tailor interactions/information given to donors on the register to ensure their priorities are addressed.

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### P131

#### Family mismatched allogeneic stem cell transplantation for myelofibrosis: Report from the chronic malignancies working party of EBMT

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Data on mismatched family donor transplants for myelofibrosis are scarce due to the risk of poor engraftment, GVHD and exclusion from trials. Outcomes from such transplants performed between 2001 and 2015 reported to the EBMT are presented. Sixty-nine patients, median age 58 (27–71) years; 44 (64%) male, 50 (74%) had primary, 18 (27%) had secondary myelofibrosis (6 from ET, 5 from PRV and 7 others) and unknown 1(2%). JAK2 V617F was mutated in 15/25. Karnofsky performance status was  $> 70\%$  in 98 %; median time from diagnosis to allograft was 41.4 (range: 0.72–213) months. The donors were predominantly male 47 (68%), median age 42 (22–75) years, HLA mismatched at 1 locus in 12 (17%) and 2 or more loci in 48 (70%). Donor-recipient serology was CMV -/- in 8 (12%)  $\pm$  in 4 (6%), +/- in 15 (22%) and +/+ in 34 (49%) missing 8 (12%). Bone marrow was used in 34 (49%) and peripheral blood in 35 (51%). The median total nucleated cell count (TNC) was  $7.5 \times 10^8/\text{kg}$  (range:  $2.3\text{--}21 \times 10^8/\text{kg}$ ) ( $n=17$ ). The median CD34+ cell dose was  $6.9 \times 10^6/\text{kg}$

(range:  $1.9-18.18 \times 10^6/\text{kg}$ ) ( $n=19$ ). patients. Conditioning was myeloablative in 48 (70%) and RIC in 21 (30%). Predominant conditioning regimens were Fludarabine, Busulphan, ATG (FBATG) and Thiotepa, Busulphan, Fludarabine (TBF  $n=33$ ). TBI was administered in 8 (12%) and T cell depletion *in vivo* in 22 (32%) and *ex vivo* in 5 (7%) patients. GVHD prophylaxis varied with post transplant Cyclophosphamide administered in 33/67 (48%) and ATG in 19/67 patients (28%). Neutrophil engraftment occurred in 53 (82%) patients at a median of 20 days (range: 11–83). Primary graft failure ensued in 8 (12%) and secondary graft failure in 4 (6%) patients at a median of 12 (range: 4.5–35) months. Eleven patients had a second allograft at a median interval of 4 (1–20) months. Responses to the first allograft censoring for a second allograft, data available in 45 patients, showed that complete remission was achieved in 35 patients (78%), 6 (13%) were never in CR and 4 (9%) were not evaluable. Relapse occurred in 8 (12%) of patients at a median interval of 3 (2.8–21.8) months. The cumulative incidence (CI) of grade II–IV acute GVHD (aGVHD) was 12% (95% CI 4–21%) and for grade III–IV aGVHD at was 5% (95% CI 3–11%). Data for chronic GVHD (cGVHD) was valid in 49 patients of whom 47% developed cGVHD. The CI of cGVHD at 2 years was 58% (95% CI 43–72%); CI of limited cGVHD was 45% (95% CI 31–59%) whereas the CI of extensive cGVHD was 10% (95% CI 2–19%). Median follow-up was 24 (95% CI 13–35) months. The 2 and 5 year OS was 53% (95% CI 40–66%) and was 40% (95% CI 23–57%). The 2 and 5 year RFS was 43% (95% CI 30–56%) and 31% (95% CI 15–47%). The 2-year CI of relapse was 20% (95% CI 10–30%). The 2 year NRM was 37% (95% CI 25–49%), which increased to 49% (95% CI 32–65%) at 5 years. Thirty patients died due to infection (16, 53%), GVHD (7, 23%), organ damage or failure (3, 10%), relapse/disease progression (1, 3%) and secondary malignancy or PTLN (1, 3%) unknown 2. There was no significant effect (univariate analysis) of recipient or donor gender, degree of HLA mismatch, CMV matching, primary or secondary MF, chronic vs advanced disease at transplant, conditioning intensity or regimen, GVHD prophylaxis with ATG or post transplant cyclophosphamide or stem cell source on overall survival. The data are encouraging for patients with myelofibrosis, with engraftment, PFS and OS being attained with limited severe chronic GVHD from family mismatched donors.

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### P132

#### Feasibility of salvage second allogeneic stem cell transplantation for disease relapse or graft failure: A single centre experience

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Despite high rates of toxicity and mortality, a second salvage allogeneic stem cell transplantation (second alloHSCT) might be an option to consider in patients experiencing disease relapse or graft failure after first alloHSCT. We retrospectively analyzed outcomes after second alloHSCT in a cohort of 30 patients (18 males and 12 females) transplanted either for disease relapse (group 1,  $n=19$ ) or graft failure (group 2,  $n=11$ ) between 2007 and 2015 in a single centre in France. Median age at second alloHSCT was 38 (range: 17–64) years. Diagnoses were acute myeloid leukemia (group 1:  $n=12$ ; group 2:  $n=3$ ), acute lymphoblastic leukemia (group 1:  $n=2$ ; group 2:  $n=4$ ), myelodysplastic syndrome (group 1:  $n=2$ ; group 2:  $n=1$ ), myeloproliferative neoplasm (group 1:  $n=3$ ; group 2:  $n=1$ ), bone marrow failure (group 1:  $n=0$ ; group 2:

$n=2$ ). Median time from first alloHSCT to second alloHSCT was 38 (range: 2.5–230) months in group 1 and 1.5 (range: 1–34) months in group 2. Graft source for the second alloHSCT were: haploidentical bone marrow (group 1:  $n=3$ ; group 2:  $n=1$ ), haploidentical PBSCs (group 1:  $n=5$ ; group 2:  $n=1$ ), cord blood (group 1:  $n=8$ ; group 2:  $n=8$ ), matched unrelated PBSC (group 1:  $n=3$ ; group 2:  $n=1$ ). At time of second alloHSCT, 11 patients were in CR and 8 presented active disease in group 1. Conditioning regimen was myeloablative in 5 patients (group 1:  $n=3$ ; group 2:  $n=2$ ), reduced intensity (RIC) in 20 cases (group 1:  $n=11$ ; group 2:  $n=9$ ). A sequential schema consisting of a combination of thiotepa, etoposide and cyclophosphamide followed by a fludarabine and busulfan-based RIC was used in 5 out of 8 patients with active disease in group 1. Sixteen patients received ATG as part of the conditioning regimen for second alloHSCT (group 1:  $n=10$ ; group 2:  $n=6$ ). All but one patient engrafted, at a median time of 18 (range: 6–51) days. Cumulative incidence of acute and chronic GVHD were  $27 \pm 17\%$  and  $42 \pm 19\%$ , respectively, 2–107) months, non-relapse-mortality (NRM) and relapse incidence (RI) were  $24 \pm 15\%$  and  $27 \pm 15\%$ , respectively, while disease-free (DFS) and overall survival (OS) were  $49 \pm 19\%$  and  $48 \pm 15\%$ , respectively, for the entire cohort. In all, 15 patients died of infections ( $n=8$ ), hematological disease ( $n=4$ ), GVHD ( $n=1$ ), hemorrhage ( $n=1$ ) and for unknown causes ( $n=1$ ). Main outcomes of patients in group 1 were: RI  $16 \pm 15\%$ , NRM  $28 \pm 18\%$ , aGVHD  $26 \pm 15\%$ , cGVHD  $56 \pm 20\%$ , DFS  $56 \pm 20\%$ , OS  $55 \pm 20\%$ , respectively. Main outcomes of patients in group 2 were: RI  $45 \pm 22\%$ , NRM  $18 \pm 24\%$ , DFS  $36 \pm 29\%$ , OS  $36 \pm 29\%$ , aGVHD  $27 \pm 23\%$ , cGVHD  $18 \pm 23\%$ . Historically, a second alloHSCT was hampered by significant morbidity and mortality. However, the advent of reduced-toxicity conditioning regimens and improved supportive care allowed to significantly improve the results of patients receiving a second alloHSCT as suggested from the above results. Therefore, a second alloHSCT could be considered as an option to rescue a certain number of patients experiencing disease relapse or graft failure, for which prognosis is very poor. Decision is to be discussed on a case-by-case basis.

**Disclosure of conflict of interest:** None.

### P133

#### Haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide for patients with high-risk hematologic malignancies

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Allogeneic hematopoietic stem cell transplantation (SCT) has been increasingly used for treatment of adult with high risk hematologic malignancies. For patients lacking an HLA-matched related or unrelated donor, unmanipulated haploidentical (haplo)-SCT is a potential alternative. Haploidentical transplantation performed with post-transplantation cyclophosphamide (PTCy)-based graft-versus-host disease (GVHD) prophylaxis has been associated with favorable outcomes for patients with acute leukemia and lymphomas. We analyzed outcomes of 45 patients with hematologic malignancies who received T-cell-replete haematopoietic stem cells and post-transplantation cyclophosphamide after myeloablative or nonmyeloablative HLA-haploidentical donor transplantation. The median age was 37 years (14–68); twelve patients were in first remission (CR1), 4 in second remission (CR2) and 29 had an active disease. The diagnosis was acute leukemia ( $n=32$ ), myelodysplastic syndrome ( $n=3$ ), Hodgkin disease ( $n=7$ ) Non Hodgkin lymphoma ( $n=2$ ) and multiple myeloma ( $n=1$ ). Median follow-up was 260 days. Stem cell source was bone marrow (BM) for 42 patients, and peripheral blood (PB) for 3. Myeloablative conditioning (MAC) was used in 37 patients and reduced intensity regimen (RIC) in 8 patients. Thirty one patients were first grafts, the others underwent previous Autologous SCT ( $n=11$ ) or MUD ( $n=3$ ). GVHD prophylaxis

consisted in PT-CY on days +3 and +4, cyclosporine (from day +5), and mycophenolate (from day +5). The median day for neutrophil engraftment was day +20 (14–29). No graft failure was observed. Chimerism was evaluable in 39 patient; on day +30 all patients had 100% donor chimerism on marrow cells. Median follow-up was 260 days. The cumulative incidence of acute GVHD grade II–IV was 22%, grade III–IV 9% and chronic GVHD 15%. One- and 2-years OS was 56.53% and 53.39 %, respectively. With a median follow-up for the surviving patients of 752 days (130–2207), the cumulative incidence of transplant-related mortality (TRM) is 22%, and the relapse-related death is 26%. Thus, we demonstrate excellent rates of engraftment, GVHD, and TRM in adult patients treated with Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide. This approach is a widely available, safe, and feasible option for adult patients with high risk hematologic malignancies, including those with a prior history of myeloablative BMT and/or those with co-morbidities or organ dysfunction, also for patients with active disease at the time of transplant.

**Disclosure of conflict of interest:** None.

#### P134

##### **Haploidentical T-cell replete transplantation with post-transplant cyclophosphamide and matched unrelated donor for patients with high-risk hematologic malignancies: A single center experience**

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It has recently been shown that T-replete allogeneic-hematopoietic stem-cell transplantation (allo-HSCT) from a haploidentical donor (haplo-ID) could be a valid option when a matched donor is not available. Unfortunately, the worldwide donor registries comprise mainly donors of Caucasian origin and patients of non-Caucasian origin have a much lower chance of finding a matched unrelated donor (MUD). The lengthy period of international search when required and the financial burden of this process are considered as additional significant limitations. At the American university of Beirut medical center (AUBMC) in Lebanon, we started the MUD program in 2011 and haplo-ID HSCT program in 2014. We report here our experience in this two groups of patients. Patients and methods: We have transplanted 21 patients from a Haplo-ID donor since 2014 and compare their outcome with the 6 patients transplanted from a MUD since 2011. The patients and transplant characteristics are listed in the Table 1. The 2 groups were comparable except for conditioning. Patients in haplo-ID group received two days of post-transplant high-dose cyclophosphamide (PT-HDCy) followed by cyclosporine A (CSA) and mycophenolate-mofetil while patients in the MUD group received pre-transplant antithymocyte-globulins and CSA starting on day-3. All patients engrafted in the MUD group, while one patient did not engraft in the haplo-ID group, the patient had refractory ALL transplanted with progressive disease, and died on day +47. The median of ANC >500/mm<sup>3</sup> was 14 days (12–20) vs 17 days (12–29) in the haplo-ID and MUD groups, respectively. Fourteen patients from the Haplo-ID group developed grade 2 acute graft-versus-host disease (aGVHD) vs one after MUD-HSCT. Two patients Haplo-ID group developed limited cGVHD and none after MUD grafts. Six patients relapsed in the Haplo-ID group vs three patients in the MUD Group. Two and three patients died from non-relapse mortality in the haplo-ID and MUD group, respectively. At the last follow-up, 13 patients are still alive in the haplo group vs 2 patients in MUD group and all of them are in CR. We conclude that T-replete Haplo-ID HSCT followed by PT-HD Cy is associated with promising results or at least comparable to patients transplanted from MUD. Haplo-ID

HSCT seemed to be safe and feasible in patients with high risk hematologic malignancies. Finally, because of the obvious advantage in rapidly finding a donor (21 haplo transplants in three years vs 6 MUD transplants in 5 years), development of haplo-ID HSCT is warranted to satisfy the regional needs.

[P134]

characteristics	N (%)	
	haplo-ID	MUD
Patients	21	6
Age at transplant median (range)	35 (22-65)	36 (24-65)
Male/female	15 (71)/ 6 (29)	1 (17)/ 5 (83)
Disease		
Acute leukemia	13 (62)	5 (83)
Lymphoma	7 (33)	1 (17)
Other	1 (5)	0 (0)
Status at transplant		
CR	14 (67)	4 (67)
PR	3 (14)	0 (0)
PD	4 (19)	2 (33)
Acute GVHD GII-III	14 (67)	1 (17)
Chronic GVHD	2 (10)	0 (0)
Progressed post transplant	6 (29)	3 (50)
Last follow up		
Alive	13 (62)	2 (33)
CR	13 (62)	2 (33)
Dead	8 (38)	4 (75)
NRM	2 (25)	1 (25)
Disease progression	6 (29)	3 (50)

**Disclosure of conflict of interest:** None.

#### P135

##### **HLA haploidentical allografting using post transplantation cyclophosphamide in high-risk and advanced MDS and AML patients aged over 50 years: Feasibility and outcome**

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Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) using T-cell-replete (TCR) grafts and post-transplantation cyclophosphamide (PTCY) provides a curative approach for patients with high-risk MDS/AML lacking a conventional HLA-matched donor. In children and adults haplo-HSCT using PTCy as GvHD prophylaxis seems to be safe with low treatment related morbidity and mortality (TRM). However, few data are available for elderly patients with advanced disease. We retrospectively analyzed the outcome of 49 patients with MDS (n=5)/AML (n=44) age 50–74 years (median age 60 years; 24 patients 50–59 years, 25 patients ≥60 years; 21 male), who underwent TCR haplo-HSCT with high-dose PTCy at our institution between January 2009 and November 2016. Disease was active in 41 patients while 8 had achieved CR. 12 patients failed previous allo-HSCT. Pre-transplantation risk factors were scored using the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) which was ≥3 in 19 patients (median HCT-CI=2, range: 0–8). A sequential therapeutic concept using either FLAMSA (n=28) or clofarabine (n=18) as cyto-reduction was used prior to reduced intensity conditioning (RIC) in all but 3 patients. RIC

consisted of fludarabine/cyclophosphamide combined with either melphalan ( $n=32$ ), busulfan ( $n=1$ ) or 4 Gy TBI ( $n=12$ ). Post-grafting immunosuppression consisted of cyclophosphamide, tacrolimus and MMF in all patients. 57% received a bone marrow graft. One graft rejection occurred. Neutrophil and platelet engraftment was achieved in 95% and 77% of evaluable patients, respectively at a median of 19 (13–89) and 33 (11–103) days. Acute GvHD grade I–III occurred in 29% of the patients whereas no grade IV aGvHD was observed. Chronic GvHD presented in 33%. It was most frequently assessed as mild to moderate (13 pts). Only 3 patients developed severe cGvHD; no GvHD related death was observed. CMV reactivated in 22 of 36 patients at risk, one patient developed CMV disease (pneumonia). No EBV reactivation or PTLD occurred. One-year TRM was 24%. 12/49 (24%) patients relapsed, three within the first 100 days after haplo-HSCT. At a median follow up of 27 months (range: 4–74 months) estimated one- and two-year overall survival (OS) was 55/46 %, respectively. When stratified by age, estimated one- and two-year OS was 65/42% in patients < 60 years and 47/47 % in patients  $\geq$  60 years ( $P=0.771/P=0.794$ ). One- and two-year progression-free survival (PFS) was 50/45%, respectively. Stratified by age estimated one- and two-year PFS was 53/41% in patients < 60 years and 47/47% in the elderly ( $P=0.836/P=0.887$ ). Unmanipulated haploidentical allografting using PTCY-based GvHD prophylaxis in high-risk MDS and AML patients aged over 50 years is safe and well tolerated resulting in acceptable TRM. A remarkable survival outcome can be achieved in elderly high-risk AML/MDS patients with significant comorbidities.

**Disclosure of conflict of interest:** None.

**P136**

**Key performance indicators to assess the quality of a collection facility: Experience of a single center**

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has evolved into an effective immunotherapy for the treatment of a variety of disorders. When patients do not have a familiar matching donor, transplant centers (TC) search for an unrelated and volunteer donor. This one must be previously evaluated by the collection center (CC) to donate

peripheral blood stem cells (PBSC) or bone marrow (BM); lymphocytes can also be asked after allo-HSCT. This work aims to evaluate our performance as CC, ensuring donor safety, quality of cell therapy products (CTP) and the accomplishment of TC requirements. We retrospectively analyzed all the requests of CTP collections sent by the Portuguese Registry from 2012 to 2016. Countries of destination, number and type of CTP were determined. We established eight key performance indicators (KPI) classified into four categories: response time; product quality; satisfaction of patients and donors; and on-site donor motivation. The intended target was defined by the mean result obtained in the first half of 2012 (excluding KPI-7). Written comments from Donor Center (DC) and TC were received by email or written in the local notebook. The donor's answers were obtained through a survey given on the collection day. A total of 349 requests were assessed: 231 PBSC, 61 BM, 16 lymphocytes and 41 cancellations; 84% were sent to Europe (98/259 to Portugal), 14% to America and 2% to Oceania; 30/41 were withdrawn by TC (14 patients died, 14 presented progressive disease and 2 had a better HLA-matched donor) and 11/41 by DC (7 donors not cleared and 4 refused). The results obtained with KPI-1, -2, -4 and -7 exceeded the intended target (Table 1). After the first KPI-1 results, we verified a positive evolution. We took an average of 3 days of delay in sending donor clearance. However, there is no holdup in the CTP delivery, as demonstrated by KPI-2. Regarding KPI-3 it is important to notice that 60% of CTP with a cell number less than requested were BM and lymphocytes; when PBSC was considered separately, the result increased (87% vs 75%). Analyzing KPI-4, 85% ( $n=11/13$ ) of the contaminated CTP were BM. Concerning KPI-5, -6 Acknowledgments and -8 Commitment, we recognize that our initial targets were too ambitious (100%). The KPI-6 shows a low number of complaints ( $n=4$ ): one due to a misreading of the request and three to communication failures; all were properly examined and rectified. A good general status was guaranteed in almost all the donors (KPI-7). The decrease of KPI-8 is due to the fact that one donor refused to proceed after three postponements of the collection date by TC. Table 1—Key performance indicators of the quality of our activity as a CC. The overall good level of our results reflects an extremely professional performance as a CC. We consider that these KPI should be continuously monitored with the purpose of earlier detect any deviation of the stated goals and assess the progress against settled strategies. We further suggest the establishment of universal indicators in order to standardize

[P136]

Category	KPI	Intended target (%)	Mean 2012-2016 (%)
Response time	1 Work-up scheduling in 5 days / Donor Clearance on the agreed date	75 / 85	82 / 89
	2 CTP delivery on the agreed date	100	100
Product quality	3 Achievement of cellular content requested	78	75
	4 Bacterial and fungal sterility	93	97
	5 Good clonogenic capacity	100	98
Satisfaction of patients and donors	6 Acknowledgments / Complaints	100 / 0	94 / 6
	7 General well-being	76	93
On-site donor motivation	8 Willingness after medical check-up / Commitment to a second collection	98 / 100	99 / 95

practices, share expertise and improve the quality of services and products provided to patients and donors. References Key Performance Indicators: Developing Meaningful KPIs, Intrafocus, 2014 Guide to key performance indicators: Communicating the measures that matter, PricewaterhouseCoopers, 2007. **Disclosure of conflict of interest:** None.

### P137

#### Myelodysplastic syndrome of donor cells

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Myelodysplastic syndrome of donor cells is a rare complication of allogeneic stem cells transplantation. Observation This is about a 50 years old Moroccan patient, with no particular previous medical history, he was diagnosed in March 2007 with a Refractory Cytopenia with Multi-lineage Dysplasia (RCMD), intermediate risk 2, according to IPSS. Two year later, the patient had a genotypical allogeneic stem cell transplant (from the bone marrow stem cell of his sister, who was 35 years old, HLA compatible). He had a reduced intensity conditioning, associating Busulfan, Fludarabine and anti-lymphocyte serum. 6 months from the ASCT, he was in complete remission with 100% donor chimerism. 4 years after the ASCT, the patient presented a progressive thrombocytopenia without any other peripheral causes. The bone marrow aspiration initially showed a Refractory Cytopenia with Multi-lineage Dysplasia. The patient was followed up during 12 months, and then a second bone marrow aspiration has shown a refractory anemia with excess blasts2 RAEB2. A cytogenetic study has every time demonstrated a female karyotype (44,XX) on 20 mitoses out is 20, and chimerism was 100% donor. The diagnosis of the myelodysplastic syndrome of the donor cells was approved. The patient was treated by Azacitidine (75 mg/m<sup>2</sup>, from J1 to J7, J1=J28). After 6 cycles, the patient was in complete hematologic response (normalization of the platelet count) and a partial bone marrow response (normalization of the blasts rate but persistence of the signs of dysplasia). He received 6 more cycles, and presented hematologic relapse (reemerging of thrombopenia). A phenotypical allogeneic stem cell transplantation was suggested. Conclusion The occurrence of MDS on the donor cells is rare. These anomalies are secondary to intrinsic factors (of donor) or extrinsic factors (of the transplant recipient). The treatment is not definitely determined.

**Disclosure of conflict of interest:** None.

### P138

#### NK-cell alloreactivity based on KIR/ligand mismatch in the donor vs recipient direction provides better graft-versus-tumor effect in patients with active hematological malignancies undergoing allogeneic T-replete haploidentical transplantation followed by post-transplant cyclophosphamide

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Haplo-SCT have been developed in the past years with very interesting results in high risk patients. GVHD prophylaxis using post-transplant cyclophosphamide (PT-Cy) recently allowed extending the use of unmanipulated Haplo-SCT. It was shown that NK alloreactivity, triggered by donor-recipient inhibitory KIR gene-gene mismatches, could lead to better

outcomes and survival in the setting of in T-cell-depleted Haplo-SCT. However, few data is available on the impact of KIR-ligand mismatch on the outcome after T-replete Haplo-SCT with PT-Cy. We thus assessed the impact of NK alloreactivity on the outcome of patients who received Haplo-SCT followed by PT-Cy. We retrospectively collected the data from patients from two centers who were treated for various high risk hematological diseases and underwent a Haplo-SCT with PT-Cy from December 2009 to December 2014. We assessed the KIR-binding epitope in HLA-C and HLA-B molecules for all patients, and we predicted NK cell alloreactivity in the donor vs recipient direction via the immune polymorphism database KIR ligand calculator, based on the KIR-ligand mismatch between donors and patients. Because disease status at the time of Haplo-SCT is one of the most important predictor of outcome, we separately analyzed two cohorts of patients: those transplanted in complete remission (CR group) and those transplanted with active disease (no CR group). Using a multivariate Cox model (adjusted by disease type, age and conditioning), we therefore evaluated the impact of NK alloreactivity on outcome in both CR and no CR groups. We analyzed 144 patients with a median age of 54y (20–74). They were mostly transplanted for lymphoma ( $n=72$ , 50%) or AML/MDS ( $n=47$ , 33%). Patients mostly received a TBI-based non-myeloablative conditioning regimen ( $n=94$ , 65%) and PBSC as graft source ( $n=91$ , 63%). Eighty one and 63 patients were transplanted in CR and in no CR, respectively. NK alloreactivity was found in 30/81 CR patients (37%) and 22/63 no CR patients (35%). With a median follow up of 30 months (12–77), CR patients had a significantly better outcome than those in the no CR group (2-year PFS 65% vs 32%, respectively,  $P<0.001$ ). In no CR patients, multivariate analysis showed that NK alloreactivity was significantly associated with reduced the risk of relapse (HR=0.25,  $P=0.019$ , Figure 1A) with no increase of both acute (HR=1.30,  $P=0.648$ ) and chronic GVHD (HR=2.61,  $P=0.232$ ), and NRM (HR=0.60,  $P=0.277$ ). This led to significantly better PFS (HR=0.41,  $P=0.014$ , Figure 1B) and a trend for better OS (HR=0.52,  $P=0.069$ ). In contrast, in CR patients, we found no difference in outcome according to NK alloreactivity for all end points (acute GVHD: HR=1.78,  $P=0.204$ ; chronic GVHD: HR=2.11,  $P=0.321$ , NRM: HR=1.69,  $P=0.313$ , relapse: HR=0.85,  $P=0.762$ , Figure 1C; PFS: HR=1.19,  $P=0.637$ , Figure 1D; OS: HR=0.83,  $P=0.672$ ). Our results suggest that NK alloreactivity provides better disease control with no increase of GVHD, especially in patients transplanted with active disease. Thus, donor selection should rely on the prediction of NK alloreactivity. This may contribute to improve outcome of these patients with high risk of relapse after transplantation, underlining the need of a specific strategy of donor search, and the promising perspective of early post-transplant NK-cell-based immunotherapy.

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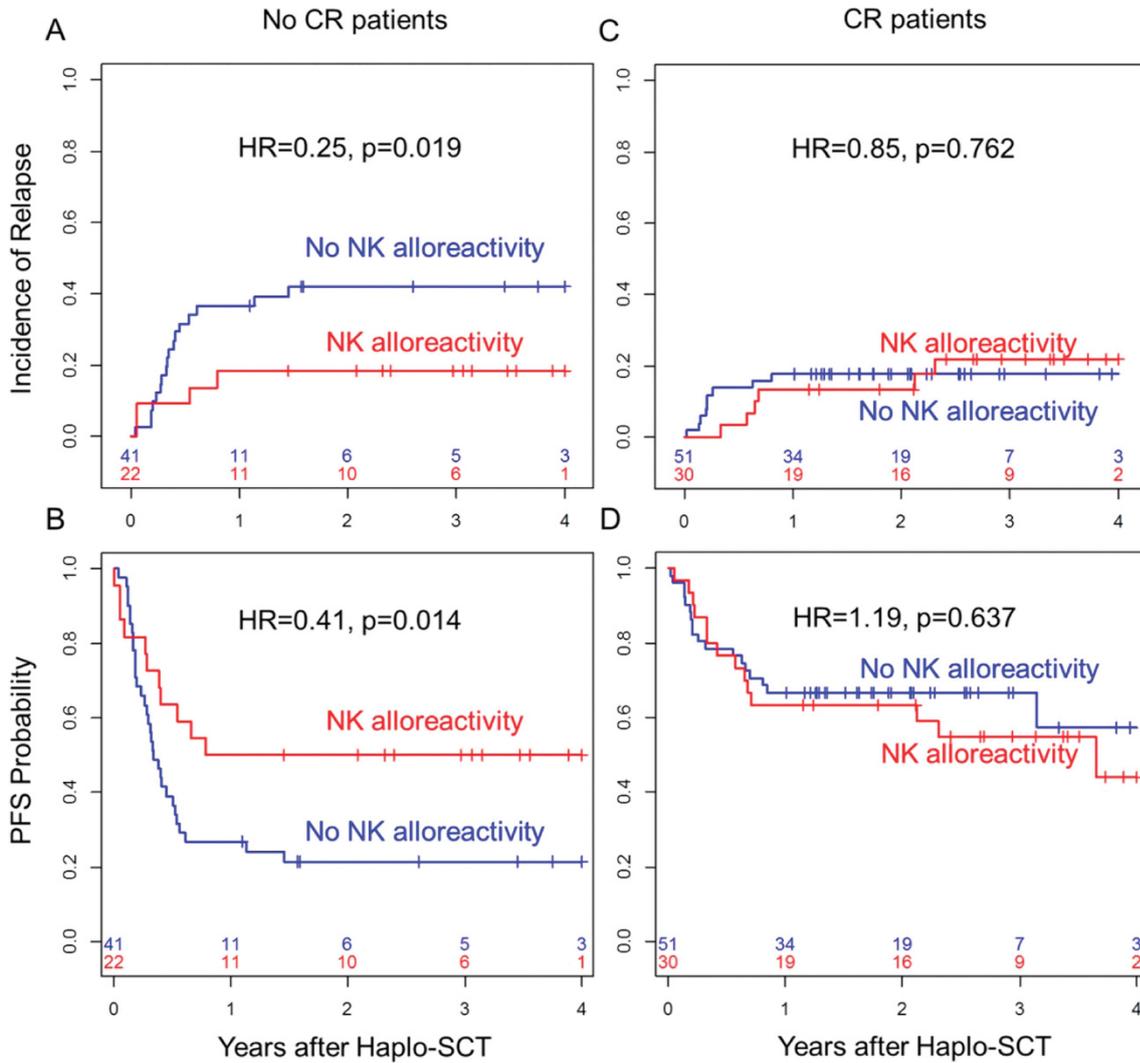
**Disclosure of conflict of interest:** None.

### P139

#### Post-transplant cyclophosphamide (PT-CY) regimen, following unmanipulated haploidentical bone marrow transplantation post myeloablative conditioning

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Haploidentical bone marrow transplantation (HAPLO-BMT) with post-transplant cyclophosphamide (PT-CY) is being increasingly used, in the last five years, for patients lacking a suitable HLA-matched donor. Genoa study (EUDRACT number: 2012-000703-32) provides for a modified GVHD prophylaxis platform compared to the original Baltimore protocol. Aim of the study: In this study we assessed outcomes in 282 consecutive patients transplanted from a haploidentical donor for haematological malignancies. All patients received a uniform GvHD prophylaxis: cyclosporine (CsA) starting on day 0, mycophenolate (MMF) starting on day +1, and post transplant Cyclophosphamide (PT-CY) 50 mg/kg, on days +3 and +5. All patients received a myeloablative conditioning consisting of thiotepa, fludarabine, busulfan (three doses  $n=116$  or two doses  $n=111$ ), or TBI, fludarabine ( $n=55$ ). The median age was 48 years (17–74); At transplant 145 (51%) patients were in remission of disease (CR1 and CR2), and 137 had an active disease (49%); all patients were first grafts. The diagnosis were acute myeloid leukemia ( $n=111$ ), myelodysplastic syndrome ( $n=31$ ), acute lymphoblastic leukemia ( $n=56$ ), myelofibrosis and myeloproliferative diseases ( $n=43$ ), non Hodgkin lymphoma ( $n=19$ ), chronic lymphocytic leukemia ( $n=9$ ) and multiple myeloma ( $n=13$ ). The median follow up was 562 days (range: 6–2241 days). The median

infused mononucleated cells was  $3.4 \times 10^8$ /kg (range: 1.1–7.7). Seven patients died before engraftment, and 21 (7%) had autologous recovery; 15 (5%) after conditioning with 2 doses of busulfan. Full-donor chimerism on day +30 was reached in 254 (90%) patients. The median day for neutrophil engraftment was day +18 (range: 13–60 days). The cumulative incidence of grade II–IV and III–IV acute GVHD (aGVHD) was 17% ( $n=49$ ) and 5% ( $n=15$ ), respectively. Two years cumulative incidence of moderate–severe chronic GVHD (cGVHD) was 13% ( $n=39$ ). Sixty one (21%) patients experienced haemorrhagic cystitis. At 3 years the cumulative incidence of non relapse mortality (NRM), relapse and relapse related death was 17% ( $n=47$ ), 32% ( $n=91$ ) and 25% ( $n=69$ ), respectively. Causes of death were infections ( $n=34$ ), hemorrhage ( $n=7$ ), GVHD ( $n=5$ ), secondary neoplasia ( $n=1$ ) and relapse ( $n=69$ ). At 4 years of follow up overall survival and disease free survival was 55.7% and 47%, respectively. At the same time overall survival rate was 73% for patients in remission and 29% for patients with active disease at transplant ( $P < 0.001$ ). In conclusion, a modified PT-Cy as GVHD prophylaxis and MA conditioning regimen followed by haploidentical BMT results in a low risk of aGVHD and cGVHD and encouraging rates of TRM and DFS.

**Disclosure of conflict of interest:** None.

**P140**

**Public events with an adequate trained medical team represent a valid option for the enrollment of new unrelated donors**

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The Italian Bone Marrow Donor Registry (IBMDR), in collaboration with ADMO (Associazione Donatori di Midollo Osseo) since 2009 has implemented, as part of the donor enrollment strategy, public enrollment events (PE). Our Donor Center (DC) has taken part to those events since the first years. One or more clinician (or trained biologist) has been present to PE to inform the potential donors, evaluate the candidates and supervise the collection of biological fluids. All the local permission were obtained. **Aim:** Aim of this study was to compare the compliance of the donor enrolled in PE with donors enrolled at our DC institutional site. We prospectively evaluated all the donors recalled for further evaluation and/or for requalification in the years 2014 and 2015 at our DC ITMI07. We defined 3 possible results for the call: 'success' (the donor was eligible and accepted to be evaluated, or only temporarily ineligible) 'not eligible' (the donor was definitively ineligible) and 'consent denied'. **Results:** A total of 286 donors were called back in the years 2014 and 2015 (16 not found). Eighty-four recalled donors had been enrolled after 2009. Among them 53 (63.1%) had been enrolled at the DC and 31 during PE (36.9%). The two populations were not different for age at the call, age at enrollment and gender (Table 1).

[P140]

		Don. Center n=53	Public Event n=31	
Age @ recall (years)	Mean	30.17	31.1	p=ns
	SD	4.91	5.96	
Age @enrollment (years)	Mean	25.55	27.52	p=ns
	SD	5.12	5.29	
Gender (M:F)		25:28	14:17	p=ns
Success		46 (86.8%)	26 (83.9%)	p=ns
Not eligible		1 (1.9%)	1 (3.2%)	p=ns
Consent denied		4 (7.5%)	2 (6.5%)	p=ns
Not found		2	2	

When evaluating the probability of obtaining a "success", no significant difference was found between the two populations: 86.8% vs. 83.9% (Chi square p=0.23). No significant difference was also found for the "not eligible" and the "consent denied" categories. Of note, when we turned to the whole 286 donor population we had called back (median age 34, range 21-55), the probability of "success" and "consent denied" were not related to donor age, and time from enrollment to recall, whereas donor ineligibility was (Spearman test p=0.02 and 0.002). Public events with the presence of an adequate trained medical team represent a valid option for the enrollment of new unrelated donors.

**Disclosure of conflict of interest:** None.

**P141**

**The search for hematopoietic stem cell unrelated donors in patients with malignant hemopathies with not-sibling matched family donor: The experience of a center**

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Unfortunately, as few as 30–35% of patients will have an HLA-identical matched sibling donor available for hematopoietic stem cell (HST) donation. The search for an unrelated donor (URD) (adult or cord blood) is often the best option for those patients lacking a suitable matched donor. Below we describe the experience with the search for an unrelated donor in our center. Between September 1995 and March 2016 the search for URD was activated for 263 patients. The median age of the patients was 46 years (range: 0.4–69), 10% were under 18 years and 60% were males. Acute myeloid leukemia (n=67), acute lymphoblastic leukemia (n=43), non-Hodgkin's lymphoma (n=60), chronic/prolymphocytic lymphocytic leukemia (n=23), Hodgkin's lymphoma (n=13), multiple myeloma (n=14), chronic myeloid leukemia (n=15), Philadelphia-negative myeloproliferative neoplasms (n=9), myelodysplastic syndrome (n=8), aplastic anemia/paroxysmal nocturnal hemoglobinuria (n=6), others (n=5). The disease status in hematological malignancies was: first CR (n=77), > second CR (n=49), PR (n=46) and refractoriness (n=82). The donor type requested at the activation of the search was an adult (n=110), umbilical cord blood (n=7) and two options (n=146). **Results:** A compatible donor was found in 197 patients (76% of the series) after a median of 44 days (range: 1–847) from the activation of the search. The degree of adult donor compatibility (not available in 7 cases) was: complete HLA identity (8/8: n=49, 10/10: n=37); an HLA difference (7/8: n=12, 9/10: n=31); lower degree of compatibility (n=13). The degree of umbilical cord blood compatibility: identity ≥ 4/6 (n=48). A total of 151 patients (57%) were transplanted, 103 from adult donor and 48 from umbilical cord blood. The median time between the activation of the search and the HST transplantation was 4 months (range: 0.7–29), being 3.2 months for acute leukemia and 5.1 months for other pathologies, and between the location of the donor and the HST transplantation 70 days (range: 5–412), being 45 days for umbilical cord blood and 76 days for an adult donor. There were 108 cancellations of the URD search (41% of the total) for the following reasons: clinical status of the patient (n=63), performing a haploidentical transplant (n=20), transplant center does not consider (N=9), norms of the registry (n=8) and loss of indication of transplantation (n=8). The median time from the beginning of the search to its cancellation was 4.5 months (range: 0.3–53). At the time of analysis, the median follow-up of the 263 patients is 17 months. The survival of the series in the 5 years is 37% and 43% for patients transplanted from URD. 76% of the searches activated in our center allowed the localization of a URD with an adequate degree of HLA compatibility. However, only 57% of the patients for whom the search was activated were finally transplanted. The most frequent cause of cancellation of the procedure was the clinical deterioration of the patient.

**Disclosure of conflict of interest:** None.

**P142**

**Sequential gain of mutations in two cases of donor cell haematological malignancy after hematopoietic transplantation revealed by whole exome sequencing.**

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(\* JS-G and CM-L have contributed equally to this work.

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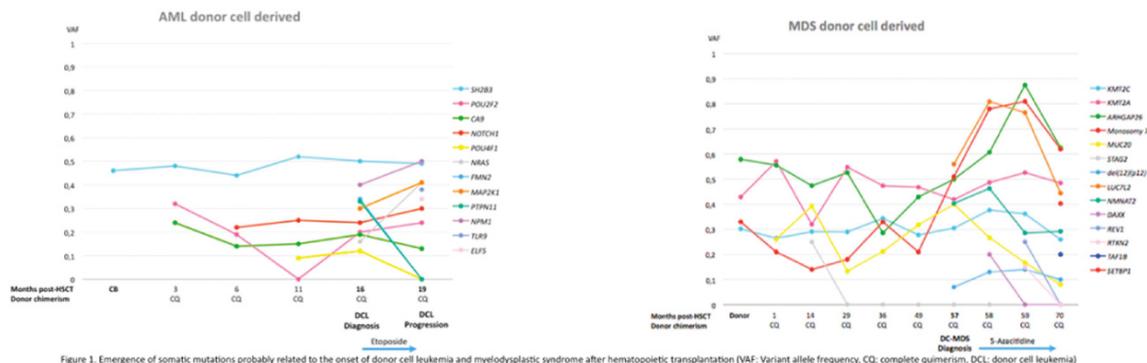


Figure 1. Emergence of somatic mutations probably related to the onset of donor cell leukemia and myelodysplastic syndrome after hematopoietic transplantation (VAF: Variant allele frequency, CQ: complete quimerism, DCL: donor cell leukemia)

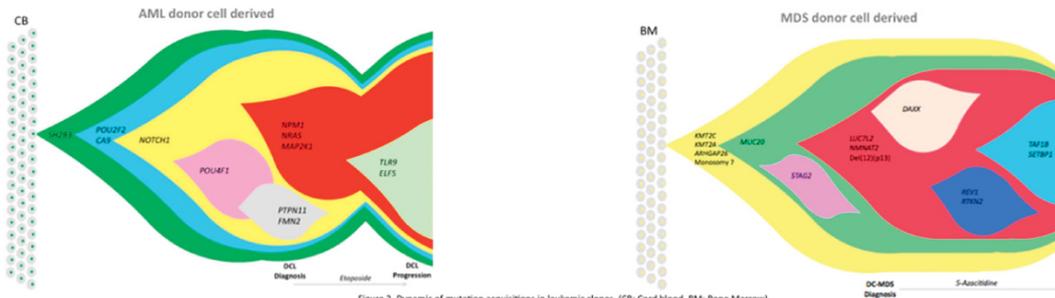


Figure 2. Dynamic of mutation acquisitions in leukemic clones. (CB: Cord blood, BM: Bone Marrow)

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The leukemic transformation of otherwise healthy donor stem cells provides a useful *in vivo* model to study the mechanisms involved in leukemogenesis. We report two cases of donor cell-derived haematological malignancy in which whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in order to study the dynamics of emergence of mutations that precede the development of donor cell leukemia (DCL) and donor cell myelodysplastic syndrome (DC-MDS). Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia-B t(1;19), who developed acute myeloid leukemia (AML) with normal karyotype, NPM1+ of donor origin 16 months after unrelated cord blood transplantation (UCBT). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45,XX,-7,del(12)(p12) of donor origin, 57 months after allogeneic BM transplantation from his HLA-identical brother. The donor also developed MDS several months later. WES (SureSelect-XT Human-exon 50Mb) was performed by next generation sequencing (Hiseq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case. In both cases non-synonymous variants in the coding regions or synonymous variants in splice regions of genes related to leukemia were selected. In addition, BM samples were matched to their SCs and to prior BM samples to identify the acquired variants. Variants meeting such criteria were evaluated with 3 functional predictor software's (SIFT, Polyphen2 and Mutation Taster). WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukemia in

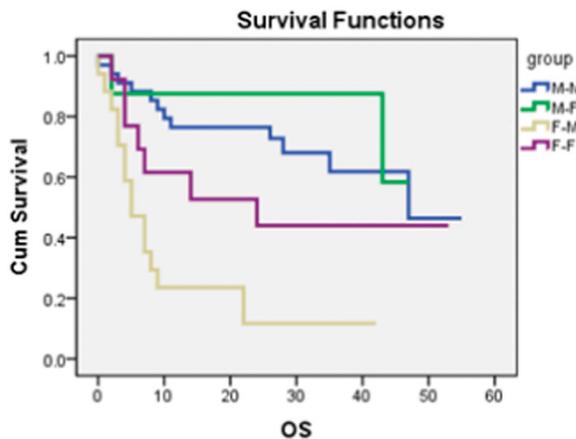
bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: SH2B3 and case 2: KMT2C, KMT2A, ARHGAP26 and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways (RAS-MAPK, pre-mRNA splicing factor, apoptosis, DNA double-strand break repair, DNA replication and so on). Mutations in leukemic subclones that disappear after chemotherapy were identified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2). The present study reveals a process of sequential clonal expansions, promoted by the acquisition of additional somatic mutations in donor hematopoietic cells. Detection of heritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing to allo-HSCT. Although the cause of donor cell-derived haematological malignancy onset seems to be multifactorial, the infusion of a SCU with pre-leukemic potential in a context of residual toxicity in recipient as a result of pre-transplant chemotherapy, a post-transplant environment characterized by a decreased immune surveillance may well have played role in these cases. The study of a greater number of DCL cases by next generation sequencing could help to understand this process and to detect new mutations involved in the emergence of AML.

**Disclosure of conflict of interest:** None.

**P143**  
**The impact of donor and recipient sex in allogeneic stem cell transplantation—single center experience (CIC 859)**  
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Allogeneic hematopoietic stem cell transplantation (HSCT) has been one of the most effective therapeutic modalities for patients with hematological malignancies and bone marrow failure syndromes. Optimal donor selection is one of the key factors to enhance the success rate of this procedure. We

retrospectively investigated whether and how donor-recipient sex affects transplantation outcomes of 73 patients transplanted between 2010 and 2015 in our center. The median age of the patients was 37 years (range: 23–51). Thirty-nine of the patients (53%) received a PBSC from a HLA-identical sibling, and 34 patients (46.5%) received PBSC from matched unrelated donor. Forty-six percent were male recipients with male donors (M–M), 11.9% were female recipients with male donors (M–F), 23.8% male recipients with female donors (F–M), and 17.8% female recipients with female donors (F–F). We performed a crosstab analysis and  $\chi^2$  tests to observe whether the donor sex affects our study population. Patients with male donor had superior overall survival and progression-free survival compared to those with female donor (66.7% vs 29.0%  $P=0.001$  for OS, and 52.3% vs 34.2%  $P=0.003$  for PFS; Cramer's  $V=0.372$ ). We further investigated how the disparity of the donor in the four groups (M–M, M–F, F–M and F–F) affects the OS, PFS and NRM. The F–M group had a worse overall and progression-free survival comparing the other groups (11% 4-year OS and 17% PFS;  $P < 0.0001$ ). This group had 27% relative increase in the non-relapse mortality compared with M–M group ( $P=0.009$ ). For M–M group there was a 2% relative increase in the subdistribution hazard of NRM compared with M–F group ( $P=0.02$ ). The F–F group and M–F group had similar subdistribution hazard of NRM (39% vs 40%  $P=0.009$ ). The incidence of acute GVHD and chronic GVHD for the groups was: 34% and 41% (M–M), 37% and 32% (M–F), 41% and 40% (F–M), 32% and 7% for the (F–F) group. The appearance of either acute or chronic GVHD did not show statistical significance regarding the OS and PFS in the groups ( $P=0.07$ ). We examined the effect of donor-recipient sex incompatibility on the outcome of HSCT in our center. Our results showed inferior OS and PFS for F–M group and a higher incidence of NRM compared with other groups. These effects might be associated with allogeneic immune responses against H-Y antigens. Key words: stem cell transplantation, donor sex, recipient sex, overall and progression-free survival [P143]



Disclosure of conflict of interest: None.

#### P144

### The Rome transplant network model compared to the Italian bone marrow donor registry activity for unrelated donor search process and transplant efficiency for hematological malignancy

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From 2011 to 2014, 66% of the 3834 patients affected by hematological malignancy searching for an unrelated donor through the Italian Registry successfully identified a suitable donor. This proportion increases up to 71% when searching for a cord blood unit was considered, corresponding to total transplant efficiency of 62%. From April 2006, the Rome Transplant Network adopted a unique policy for the identification of a potential alternative donor, following a hierarchical selection that considered as first choice a volunteer unrelated donor, secondly a cord blood unit and last a haploidentical related donor. Before starting the unrelated donor search, a preliminary query through the Bone Marrow Donor Worldwide database was performed for all the patients referred to the Rome Transplant Network. Based on the low resolution HLA typing (A, B and DRB1) it was possible to arbitrarily assign a good or poor score that might predict the identification of a full matched (8/8 A, B, C and DRB1) donor. Therefore, aims of the present study were to assess the utility of the preliminary query and the impact of the use of high resolution HLA typing since the starting of donor search on the timing for the unrelated donor identification. Moreover, the final aim was of comparing donor identification and transplant efficiency between the National Registry, that considers only the unrelated donor and the Rome Transplant Network, whose policy includes also haploidentical donor as third choice in the donor search process. At Rome Transplant Network 79% out of 417 adult patients met criteria of a good preliminary query corresponding to a matched unrelated donor identification in 50% of cases vs only 12.5% for patients with poor preliminary query. Our policy led to 78% and 74%, respectively, of alternative donor identification and transplant efficiency, significantly higher than the corresponding data of 71% ( $P=0.007$ ) and 62% ( $P < 0.0001$ ) reported by the National Registry. Moreover, the median duration of search process for MUD identification has been significantly reduced by the use of HR HLA typing patient at the start of the formal search activation from 88 (range: 1–1016) to 66 (range: 8–905) days at IBMDR ( $P < 0.001$ ) and from 61 (3–765) to 41 days (20–321) at RTN ( $P < 0.001$ ). In conclusion, the preliminary query represents a useful tool to address the search towards the best donor choice and to perform transplant in adequate time. Moreover, the timing of donor identification has been significantly reduced with the use of high resolution typing at the start of donor search. A search and selection donor policy should be basically established and should include the haploidentical donor to improve the transplant efficiency.

Disclosure of conflict of interest: None.

#### P145

### The treatment outcome of hematopoietic stem cell transplantation according to donor types in elderly patients with acute myeloid leukemia

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The long term prognosis of elderly acute myeloid leukemia (AML) patients remains poor. Advances in the uses of

alternative donors and reduced intensity conditioning regimens have extended the use of allogeneic hematopoietic stem cell transplantation (HSCT) to a wider number of patients. However, few studies have reported data on the efficacy of HSCT from alternative donors in elderly AML patients. We retrospectively analyzed the transplantation outcome in 93 consecutive elderly AML patients aged >60 years who received HSCT (2005–2015) at the Catholic Blood and Marrow Transplantation Center. Donor types were autologous ( $n=18$ ) or HLA matched related (MRD,  $n=28$ ), unrelated (MUD,  $n=22$ ), or haploidentical ( $n=25$ ). For graft-versus-host disease (GVHD) prophylaxis, methotrexate and cyclosporine (MRD) or tacrolimus (MUD/haploidentical donor) were used. MUD and haploidentical donors were given antithymocyte globulin. The median age was 63 years, with 23 patients (25%) >65 years. Intermediate- or adverse cytogenetic risk was observed in 91% of patients. With a median follow-up of 44.7 months, overall survival (OS) and disease-free survival (DFS) at 3 years after transplantation were 37% and 38% for autologous, 40% and 35% for MRD, 67% and 62% for MUD, and 67% and 67% for haploidentical HSCT, respectively. The 3-year relapse was significantly higher for autologous HSCT compared to allogeneic HSCT (40% vs 14%,  $P=0.012$ ), while it was similar among allogeneic donors: MRD, 13%; MUD, 14%; haploidentical, 15% ( $P=0.925$ ). The 3-year non-relapse mortality (NRM) for MUD (24%) or haploidentical donor (18%) HSCT was comparable to that of autologous HSCT (22%), while it was relatively higher for MRD HSCT (52%,  $P=0.056$ ). Of the 75 patients receiving allogeneic HSCT, the 1-year cumulative incidence of moderate to severe chronic GVHD was significantly increased for MRD (64%) compared to alternative donor HSCT (35%,  $P=0.001$ ). In multivariate analysis, patient age (HR 0.8, 95% CI 0.8–1.0,  $P=0.005$ ) and donor type (HR 3.5 95% CI 1.0–13.0,  $P=0.056$  for MUD; HR 6.2, 95% CI 1.7–22.6,  $P=0.006$  for MRD compared to haploidentical donor) were significantly associated with the cumulative incidence of moderate to severe chronic GVHD, while female-to-male HSCT showed a borderline significance (HR 2.1, 95% CI 0.9–4.7,  $P=0.075$ ). Incidence of acute GVHD was similar according to donor type. In the multivariate analysis for NRM, patient age (HR 1.4, 95% CI 1.1–1.6,  $P=0.001$ ), MRD (HR 4.5, 95% CI 1.4–14.4,  $P=0.011$ ), and hematopoietic cell transplantation-comorbidity index high risk (HR 6.4, 95% CI 2.3–17.5,  $P=0.001$ ) were significantly associated. In conclusion, our results showed significantly higher relapse rate for elderly AML patients receiving autologous HSCT compared to allogeneic HSCT, responsible for the lower survival rate in autologous HSCT. We observed that NRM rate for MUD and haploidentical donors for elderly AML patients were lower than expected and similar to autologous HSCT. Relatively higher incidence of NRM for MRD HSCT seemed responsible for the low long term DFS. These results suggest a need for strengthening of GVHD prophylaxis in MRD HSCT for elderly AML patients. Our results suggest a potential role of alternative donor HSCT to improve long term survival rates in elderly patients with AML.

**Disclosure of conflict of interest:** None.

#### P146

##### **Unrelated transplant for severe aplastic anemia (SAA): Long term results and risk factor analysis for overall survival**

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For patients with SAA, Transplantation from an unrelated donor (UD) is usually considered after failure of at least one course of immunosuppression. This strategy is based on a relatively high risk of complications for UD transplant recipients, such as graft rejection, graft-versus-host disease (GVHD) and infections. However, the outcome of unrelated donor transplants has significantly improved in recent years, due to better donor selection, conditioning regimen

optimization and better supportive care. The authors describe results from 51 patients with SAA who receive unrelated allogeneic transplants in a single reference institution from 1997 to 2014. Data was retrieved from the center databasis and There were 30 females and 21 males. Median age was 15 years old (0–47). Median total number of cells infused was  $3.4 \times 10^8$ /kg. 61% of the patients have received more than 50 transfusions previously. Conditioning regimen were: CY 120 + TBI 1320 ± ATG in 16 (31%) patients, Bu 12 mg/kg + Cy 120 + ATG in 18 (35%), and Fludarabine + Cy + ATG in 8 (16%), Fludarabine, Cy + TBI 200 in 9 (18%) patients. Stem cell source was marrow in 84%, cord blood in 13% and peripheral blood in 3% of patients. Transplants were full matched in 32 (62%) patients, had one mismatch (out of 12) in 12 (24%) and 2 mismatches in 7 (14%) patients. Engraftment was complete as evaluated by donor chimerism at day 30 and 100 post transplant in 36 patients (71%), partial in 4 (8%) and graft failure was observed in 9 (18%) patients. Acute GVHD grade II–IV was seen in 9 patients (18%) and NIH moderate to severe chronic GVHD was seen in 8 (16%) patients. Median overall survival was 328 days (4–4287) and estimated 5 years overall survival was 55%. Risk factors for survival identified were: HLA mismatch and stem cell sources other than marrow. Unrelated transplants are a feasible salvage therapy for patients with SAA refractory to immunosuppression, being HLA compatibility and marrow stem cell source factors with a positive impact on survival.

**Disclosure of conflict of interest:** None.

#### P147

##### **Use of haploidentical stem cell transplantation continues to increase, the 2015 European society for blood and marrow transplant activity survey report**

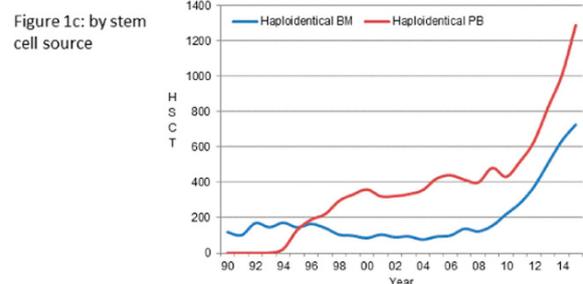
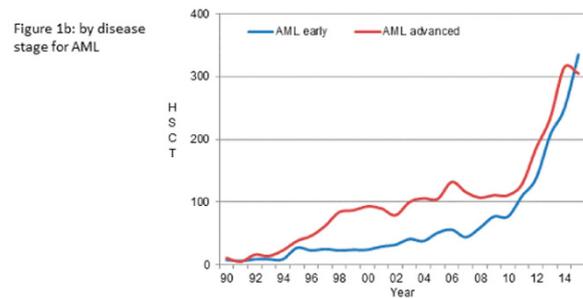
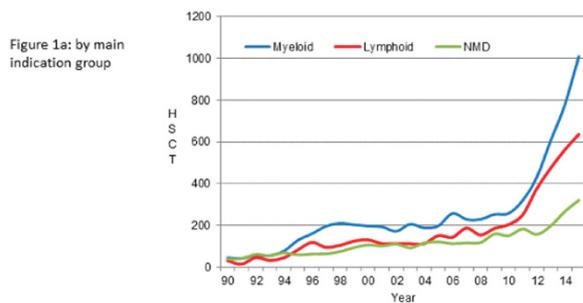
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Hematopoietic stem cell transplantation (HSCT) is an established procedure for many acquired and congenital disorders of the hematopoietic system, including disorders of the immune system, and as enzyme replacement in metabolic disorders. The annual activity survey of the EBMT describes the status of HSCT in Europe and affiliated countries and has become an instrument used to observe trends and to monitor changes in technology use. Teams were invited to report their transplant activity for 2015 by indication, stem cell source and donor type using a single paged survey. A record number of 42 '171 HSCT in 37 '626 patients (16 '030 allogeneic (43%), 21 '596 autologous (57%)) were reported by 655 centers in 48 countries in 2015. Trends include continued growth in transplant activity during the period 2005 and 2015, with

the highest percentage increase seen in middle income countries (allo 209%, auto 215%), and the lowest in very high income countries (allo 64%, auto 28%), for both allogeneic and autologous HSCT. In contrast the absolute growth is highest in the very high income countries (growth allo rates 114 transplants per  $10 \times 10^6$  inhabitants, auto rates 85 for very high income countries; allo rates 35, auto rates 38 for middle income). Main indications for HSCT were myeloid malignancies 9 '413 (25%; 96 % allogeneic); lymphoid malignancies 24 '304 (67%; 20% allogeneic); solid tumors; 1 '516 (4%; 3% allogeneic); and non-malignant disorders; 2 '208 (6%; 90% allogeneic). Remarkable is a decreasing use of allogeneic HSCT in CLL from 504 patients in 2011 to 255 in 2015 and is most likely due to the development of potentially very effective CLL drugs. Use of haploidentical donors for allogeneic HSCT continues to increase 2 '012 in 2015; a 291% increase since 2005. The highest growth is seen in myeloid malignancies 1 '008, with lymphoid malignancies 636, nonmalignant disorders 316 and 52 others. In AML, haploidentical HSCT increases similarly for patients with both advanced disease and those in CR1. Both marrow and peripheral blood is used as stem cell source for haploidentical HSCT with higher numbers reported for the latter. This year's activity survey shows continued increase in the use of haploidentical HSCT across Europe within the main indication groups and cell source. It reflects in a timely manner current trends in stem cell transplantation and is an essential tool for health care planning and health policy makers. Figure 1: Trend in haploidentical HSCT in Europe 1990–2015: Figure 1a: by main indication group Figure 1b: by disease stage for AML Figure 1c: by stem cell source.

[P147]



**Disclosure of conflict of interest:** None.

## Graft-versus-host disease—preclinical and animal models

**P148**  
**Previously published**

**P149**  
**Immune modulation of activated lymphocytes by human bone marrow mesenchymal stromal cells derived exosomes**

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Human Bone Marrow Mesenchymal Stromal Cells Derived Exosomes (hbmMDEs) are small membrane vesicles secreted from Mesenchymal Stromal Cells that may serve as a vehicle for protein, mRNA and microRNA (miRNA) transfer to distant cells; affecting gene expression, proliferation, and differentiation of the recipient cells. Therefore, MDEs may possess some of the immunoregulatory properties of their parental cells. In the present study we aim to explore the immunomodulatory function of MDEs and understand the molecular mechanisms enabling it. For this purpose, we co-cultured hbmMDEs with activated human lymphocytes. Using ultracentrifugation, HbmMDEs were isolated from expanded human bone marrow derived Mesenchymal Stromal Cells (hbmMSCs). Using EM and Zeta sizer, particles were shown to be in the range of 80–120 nm. PHA activated human Peripheral blood lymphocytes (PBLs), R-848/IL2 activated B cells and Anti CD3/CD28 activated T cells were co cultured with purified MDEs. Cell proliferation was tested using thymidine incorporation assay. We found that exosomes derived from  $1 \times 10^3$  to  $1 \times 10^6$  MSCs exhibited a dose-dependent inhibition of lymphocyte proliferation. Exosomes derived from  $1 \times 10^6$  mesenchymal stromal cells co cultured with PHA activated PBLs, activated B cells and activated T cells showed proliferation inhibition of 53% ( $P \leq 0.001$ ), 34.37% ( $P \leq 0.05$ ) and 47.41% ( $P \leq 0.01$ ), respectively. In order to understand the molecular mechanism behind the immunomodulatory effect of MDEs, we have profiled MDE's miR content using Illumina HiSeq 2500 platform and we are currently profiling co cultured activated lymphocytes mRNA content using Next-Generation Sequencing System, Illumina. Preliminary results demonstrate some higher abundance of specific MSCs derived miRs in the MDEs. hbmMSCs have been shown to serve as immune modulators in patients with acute and chronic graft versus host (GVHD). In the future, MDEs may provide an alternative therapy for GVHD. Compared with bmMSCs, MDEs are more stable, have no risk of aneuploidy or ectopic proliferation and have less probability of immune rejection. Additional studies are needed to explore the applicability of MDEs to serve as modulators of the immune response.

**Disclosure of conflict of interest:** None.

**P150**  
**Immunomodulatory effect of polyphenols obtained from olive oil in acute graft versus host disease**

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Graft-versus-host disease (GVHD) is the major complication after allogeneic haematopoietic stem-cell transplantation

(HSCT). Extra virgin olive oil (EVOO) is a source of phenolic compounds such as glycoside oleuropein, hydroxytyrosol and tyrosol. Olive oil polyphenols have shown antioxidant, immunomodulatory, antiproliferative, anti-apoptotic and anti-inflammatory properties that might be useful in the prophylaxis and treatment of GVHD. Polyphenolic extract (PE) of EVOO was obtained by the method described by Vazquez Roncero *et al.* with some modifications. Briefly, fifty grams of EVOO (Oleoestepa, Seville, Spain) was extracted with methanol/water (80:20, vol/vol, 125 mL). The mixture of EVOO, methanol and water was decanted and the methanolic extract was concentrated and lyophilized. Then, the effect of PE in cell viability and activation of T lymphocytes from healthy donor's Buffy Coats either resting or activated with antiCD3 plus antiCD28 was analyzed by flow cytometry after staining with 7AAD, Anexin-V and CD25. Proliferation assays were performed with PKH and the quantification of IL-2, IL-4, IL-6, IL-10, TNF- $\alpha$  and IFN- $\gamma$  cytokines in cell culture supernatants with BD Cytometric Bead Array (CBA). Signaling pathways were analyzed by Western Blot. Finally, in a mouse model of acute GVHD (C57BL/6 in BALB/c), mice were randomized into two experimental diet groups: standard diet (2014S Harlan Laboratories) and standard diet (2014S Harlan Laboratories) supplemented with 600 ppm of PE obtained of EVOO. The severity of GVHD was assessed by a scoring system described by Cooke *et al.* that incorporates five clinical parameters: weight loss, posture (hunching), activity, fur texture, and skin integrity. PE did not affect T cell viability. By contrast, PE decreased T-cell activation and proliferation of T-lymphocytes stimulated with antiCD3 plus antiCD28. In addition, there was a decreased production of Th1 (IFN $\gamma$ , IL-2 and TNF) and Th2 cytokines (IL-4, IL-6 and IL-10) in the presence of PE. Regarding the signaling pathways analyzed, PE inhibited phosphorylation of Akt and nuclear translocation of NFkB in activated T cells. In the mouse model of acute GVHD, animals which received the PE supplemented diet had an increased survival as compared to mice receiving a standard diet. Also, GVHD incidence was significantly lower among mice receiving the PE supplemented diet as assessed by both the presence of GVHD signs as well as pathological examination. Polyphenols obtained from EVOO are an important immunomodulatory agent capable to reduce the proliferation and activation of activated T cells and the production of proinflammatory cytokines. In a mouse model of acute GVHD, PE supplemented diet reduced the incidence and severity of the disease and increased the survival of mice.

**Disclosure of conflict of interest:** None.

**P151**

**Immunomodulatory effects of extracorporeal photopheresis in patients with GvHD**

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Graft-versus-host disease (GvHD) is a leading cause of post-allogeneic haematopoietic stem cell transplantation (HSCT) morbidity and mortality (1). Extracorporeal photopheresis (ECP) is an alternative therapeutic strategy that appears to act in an immunomodulatory fashion, potentially involving regulatory T lymphocytes and dendritic cells in patients who are refractory to steroids. Dendritic cells (DCs) are the most important antigen-presenting cells, playing a pivotal role in T-cell function and in the link between innate and adaptive immunity. Moreover, DCs are also critical mediators of immune tolerance and energy. They can be divided into two major subsets, plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) which have distinct functions. pDCs play a pivotal role in peripheral tolerance through generation of regulatory T (Treg). On the other side mDCs promote, as well as pDCs, Th2 and Th0/Tr1 responses (1–4). Our study was performed to understand the mechanism of action involved in immunomodulatory effect of ECP. As the modulation of DCs and Tregs number and function (7, 8) may be a central mechanism of ECP in maintaining self-tolerance, down-regulating immune responses, and limiting inflammation (9). Eight patients affected by GVHD were included in this pilot study. In ECP apheresed mononuclear cells are exposed to 8-methoxypsoralen and UVA radiation. After this photoactivation, which induces DNA damage and apoptosis, the cells exposed are re-infused into the patient inducing an immunomodulatory effect. All patients or their legal guardians gave their consent for this study. A sample of peripheral blood (PB) (basal condition), a sample of apheresis pre-UVA photoactivation (pre-PA) and a sample of photoactivated apheresis (PA) were collected at the first day of ECP and every week for the first month of treatment. Circulating DCs, mDCs (CD14/16-CD85+CD33+), pDCs (CD14/16-CD85+CD123+) and Tregs (CD4+CD25+FOXP3+) were directly enumerated and phenotypically characterized. The assays were performed at day+1,+8,+15,+21,+30 Data are expressed as mean  $\pm$  s.d. of absolute number of cells/ $\mu$ L. At day +1 there were no differences in the absolute number of both mDCs and pDCs between Pre-PA and PA. Consequently there were no differences between PB and PA. From day +8 till +30 we observed an increase of these two cellular populations at every date of treatment. Comparing the basal PB of day +1 vs day +30 we observed an increment of 40% and 120%, respectively for mDCs and pDCs (mDC from 11247 cell/ $\mu$ L to 15742 cell/ $\mu$ L; pDC from 6983 cell/ $\mu$ L to 15263 cell/ $\mu$ L). Comparing the basal PB of day +1 vs day +30 we observed an increment of 115% of Tregs (from 4257 cell/ $\mu$ L to 9142 cell/ $\mu$ L) while we observed a median increment of 34% calculated between Pre-PA and PA of each day of treatment from day 1 to day +30. No firm conclusions can be drawn from a clinical point of view, however a biological effect has certainly highlighted. In particular no substantial differences in basal PB mDC or pDC emerged during the first month of treatment while a significant increase of mDC and pDC can be observed since day +15 following UVA photoactivation. Regarding TREGs we observed an increment of 115% of Tregs between PB from day +1 to day+30 and a median increment of 34% calculated between Pre-PA and PA of each day of treatment.

**Disclosure of conflict of interest:** None.

[P151]

	mDC			pDC		
	PB	PA	$\Delta\%$	PB	PA	$\Delta\%$
day+1	15997	13381	-16%	3923	19853	406%
day+8	16350	45089	176%	17909	41282	131%
day+15	10021	44059	340%	14087	93153	561%
day+21	8614	50315	484%	16265	53146	227%
day+30	15742	88121	460%	10402	152575	136%

	mDC			pDC		
	Pre-PA	PA	$\Delta\%$	Pre-PA	PA	$\Delta\%$
day+1	29361	13381	-54%	37079	19853	-46%
day+8	46128	45089	-2%	37136	41282	11%
day+15	43286	44059	2%	39882	93153	137%
day+21	36052	50315	40%	46920	53146	13%
day+30	64287	88121	37%	62797	152575	143%

**P152****Impact of Th17 cells on xenogeneic graft-versus-host disease**

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Acute graft-versus-host disease (GVHD) is a severe complication of allogeneic hematopoietic stem cell transplantation. Its pathophysiology is complex and not yet fully understood. In particular, the impact of Th17 cells on murine acute GVHD has yielded conflicting results, while demonstration of increased levels of Th17 cells at the site of acute GVHD provided only indirect evidence of their involvement in humans. Here, we assessed the potential implication of Th17 cells in a humanized mouse model of xenogeneic GVHD (x-GVHD). Methods: X-GVHD was induced by infusing human peripheral blood mononuclear cells (PBMCs) into NOD-scid IL-2R $\gamma$ null (NSG) mice given 2.5 Gy total body irradiation 1 day prior transplantation. Th17 cells were generated by culturing naive CD4<sup>+</sup> T cells with anti-CD3/anti-CD28 coated beads under Th17-skewing cytokines (TGF- $\beta$ 1, IL1- $\beta$ , IL-6, IL-21, IL-23, neutralizing anti-IL-2 and anti-IFN $\gamma$  antibodies) in hypernatremic conditions (NaCl 40 mM). **Results:** After 8 days of culture, a median of 21.75% of IL-17A<sup>+</sup> cells was obtained. We confirmed the expression of IL-17A, RORC and IL-23R by these cells by RT-qPCR. We next assessed the co-injection of human PBMCs (1.10<sup>6</sup>) with *in vitro* differentiated cells under Th17 skewing conditions (1  $\times$  10<sup>6</sup>) (co-injection group, N=20), in comparison with the injection of PBMCs alone (2  $\times$  10<sup>6</sup> cells, PBMCs group, N=17). We observed higher x-GVHD score (p60%) of cells expressing both IL-17A<sup>+</sup> and IFN $\gamma$ <sup>+</sup> cells (Th17/Th1-like phenotype) among CD4<sup>+</sup> IL-17A<sup>+</sup> cells while co-injected mice had higher blood concentration of IL-17A (P=0.026) than PBMC mice. These results demonstrate that addition of Th17 cells worsened x-GVHD confirming their role in acute GVHD pathogenesis.

**Disclosure of conflict of interest:** None.

**P153****Previously published****P154****Metagenomic analysis of gut microbiota in pediatric allogeneic stem cell transplantation underscores marked differences in patients with GVHD**

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Although survival from allogeneic stem cell transplantation (HSCT) has significantly improved, acute Graft-versus-Host Disease (GvHD) remains a major cause of death. Intestinal dysbiosis has been associated with acute gastrointestinal GvHD and poor outcome after HSCT. We reported a correlation between microbiota (GM) composition and short chain fatty acid (SCFA) production and GvHD in transplanted children.<sup>1</sup> To assess how the metabolic pathways of GM change during transplantation and identify modulators of immune response, we perform first longitudinal metagenomic analysis in children undergoing HSCT. 8 patients (pts) (6male; mean age: 10y) with hematologic malignancies (7 ALL, 1 AML), who received busulphan-based myeloablative conditioning and T-cell replete bone marrow graft were enrolled. Pts were prospectively enrolled in a protocol with at least 3 specimens fecal samples collected: one before and two after HSCT, in order to

build a proper trajectory. GvHD prophylaxis was cyclosporine for 3 pts receiving a matched related donor and cyclosporine, short-term MTX and ATG for 5 pts receiving a matched unrelated donor. Non-GvHD and GvHD patients had similar exposures to antibiotics during the stool collection. Of these pts, 50% developed GVHD within the first 100 days. We applied shotgun metagenome sequencing to total fecal DNA from samples collected. Functionalities were assigned by reads mapping at different levels of the KEGG database.<sup>2</sup> Relative abundance was calculated and statistical analysis was performed. According to our findings, core functional profiles were overall conserved through the time-points in all patients (Figure 1A), in contrast to the phylogenetic profiles behavior, this finding confirming the overall redundancy of gut microbiome core functionalities. Analyzing the single metabolic pathways in subjects who developed GvHD, we found in the pre-HSCT period a higher relative abundance of nucleobasis (purine and pyrimidine) metabolism (P < 0.05) and branched-chain amino acids biosynthesis (P < 0.05). Functions related to the production of branched-chain amino acids are involved in the biosynthesis of the cell wall of Gram-negative bacteria, microorganisms including subgroups with well know opportunistic pro-inflammatory. In addition, post-HSCT samples of GvHD patients showed a lower abundance of genes involved in polysaccharides metabolism, as glycan biosynthesis and glycosaminoglycan degradation (P < 0.05) (Figure 1B). Glycosaminoglycan degradation activity gets bacteria able to survive during extreme situations, as fasting using mucus polysaccharides as energy source, contributing to maintain a mutualistic composition of GM and SCFA production by the saccharolytic functions of the endogenous mucus polysaccharides. This study detects functional peculiarities in the GM of non-GvHD pts. The gut metagenome configuration of non-GvHD patients is structured to derive SCFA after HSCT. The production of these metabolites promotes peripheral regulatory T-cell generation<sup>3</sup>, potentially explaining the protective role of GM from GVHD.

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**Disclosure of conflict of interest:** None.

**P155****Protection of intestinal epithelial cell damage with aryl hydrocarbon ligand reduces intestinal GVHD in mice**

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Although intestinal epithelial cells (IECs) are crucial regulators of barrier function and immune homeostasis, they also facilitate inflammation in exaggerate responses to pro-inflammatory mediators by pretransplant conditioning regimen, which plays a critical role in amplifying graft-versus-host disease (GVHD). Thus inhibition of the converting to pathogenic IECs by conditioning may represent a novel approach to inhibit GVHD. Aryl hydrocarbon receptor (AhR) is the ligand-activated transcription factor which has the ability to mediate the biochemical, metabolic, and toxic effects of environmental chemicals. Recently, it has been demonstrated that AhR is an important regulator of cell development, differentiation, and function of both innate and adaptive immune cells. The ability of AhR is induced by respond to endogenous ligands generated from the host cell, diet, and microbiota. Here, we investigated the regulatory role of AhR in IECs under

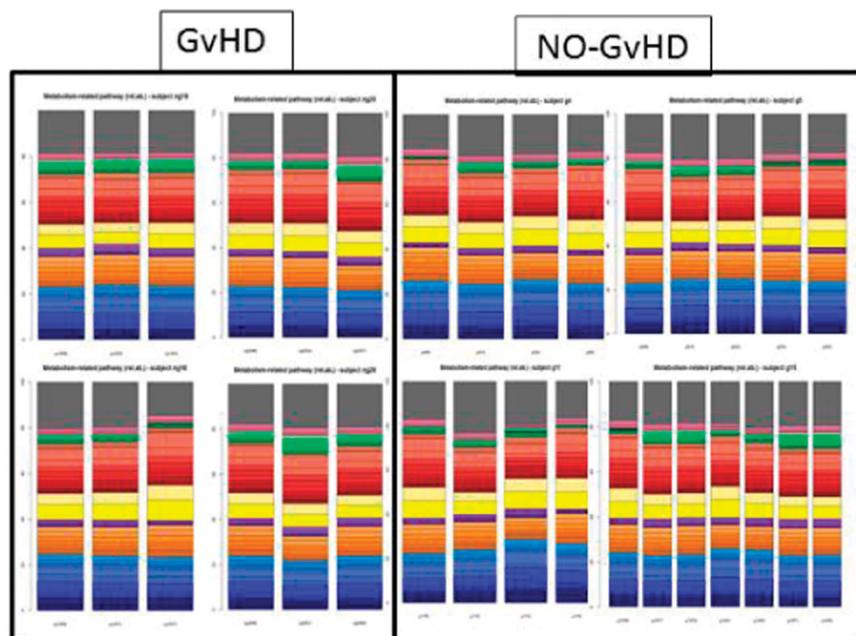


Fig. 1A

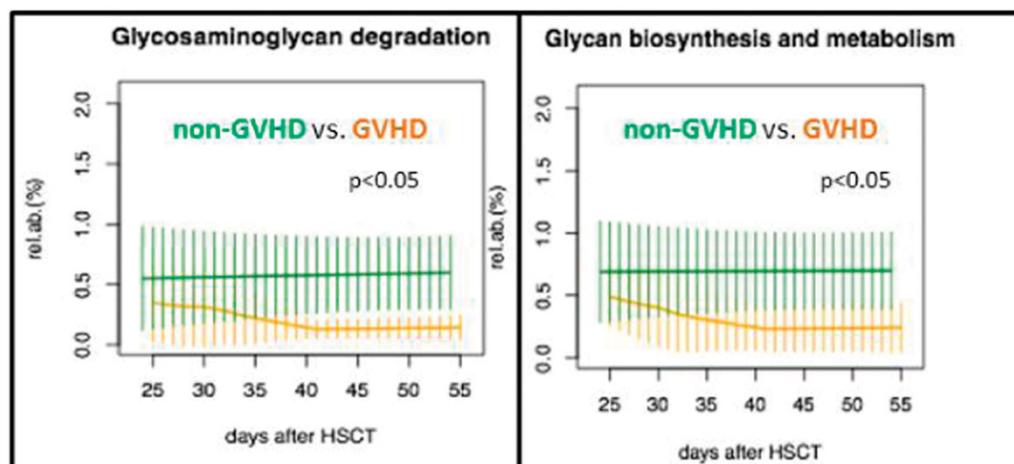


Fig. 1B

inflammatory responses and its therapeutic activity for modulation of GVHD. AhR and CYP1A1 expression in mouse IECs were determined by real-time PCR. Mouse IECs were pretreated with endogenous AhR ligands L-kynurenine (L-Kyn, 300 mM) or PBS for 6 h and then stimulated with LPS or IL-1 $\beta$  for 24 h. Cytokine levels were measured using the mouse Flex-Set cytokine bead array or real-time PCR. B6D2F1 (H-2b/d) recipients were administrated L-Kyn daily by i.p. injection for 3 days. Then the recipients were lethally irradiated and transplanted with  $5 \times 10^5$  TCD-BM plus  $2 \times 10^6$  T cells from B6 (H-2b) donor. Mice were monitored every other day for survival and clinical score. Colons were collected and stained with hematoxylin and eosin (H&E) for histopathological scoring. We found that AhR was constitutively expressed in the mouse IECs. CYP1A1 (an AhR target gene) was significantly increased by treatment of L-Kyn under un-stimulatory condition. We further observed that L-Kyn completely abrogated IL-1 $\beta$ -mediated IL-6 or LPS-mediated TNF- $\alpha$  expression in IECs. Administration of BDF1 recipient mice with L-Kyn before transplantation significantly reduced the lethality and severity of GVHD. Histopathology clearly revealed that treatment of L-Kyn inhibited intestinal GVHD. Our results demonstrate

that 1) AhR is constitutively expressed in IECs, 2) treatment of endogenous ligand L-Kyn induce AhR activation in the steady status, 3) AhR activation blocks conversion of the epithelial cells into pathogenic cell type, and 4) pre-administration of AhR ligand reduces GVHD. Our study suggests that activation of AhR pathway in IECs before allogeneic hematopoietic stem cell transplantation (HSCT) is a possible strategy to reduce intestinal GVHD.

**Disclosure of conflict of interest:** None.

**P156**  
**Simvastatin ameliorates graft-vs-host Disease by regulating angiotensin-1 and angiotensin-2 in a murine model**

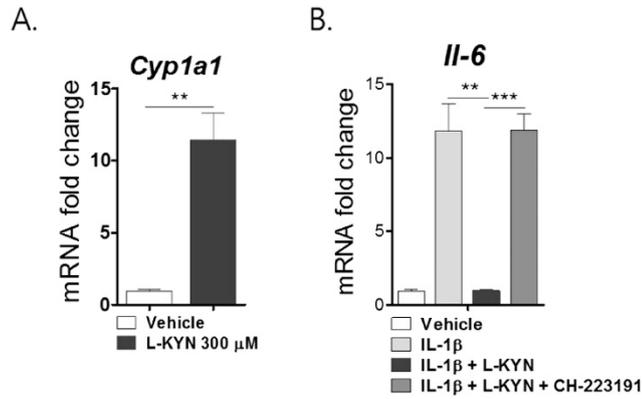
P Zheng,\* Q-L Wu<sup>#</sup>, B-B Li<sup>#</sup>, P Chen<sup>#</sup>, D-M Nie<sup>#</sup>, J Fang<sup>#</sup>, L-H Xia<sup>#</sup> and Mei Hong<sup>#</sup>

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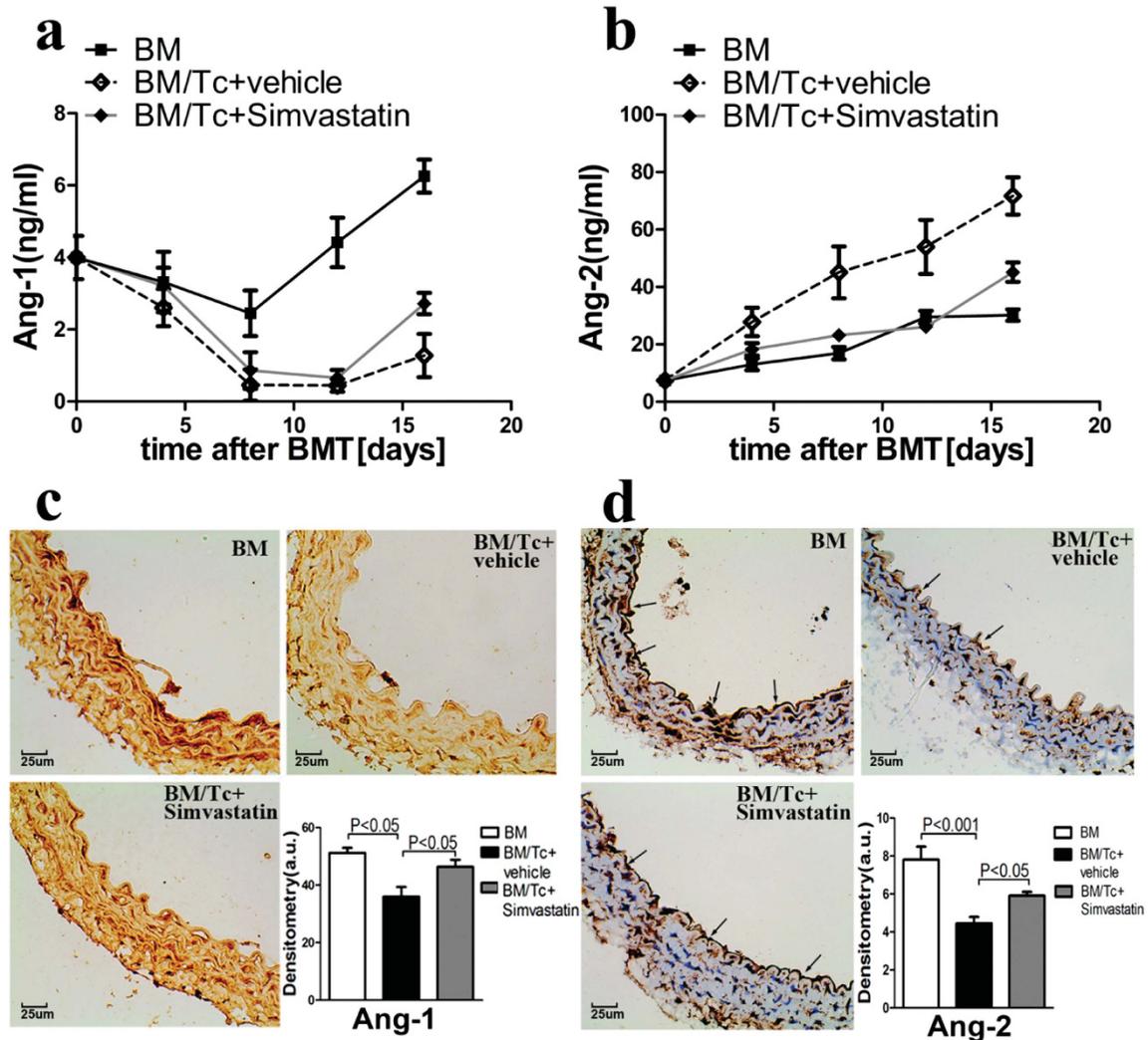
Angiotensins play an important role in vascular endothelial function. Endothelial damage is an important pathogenesis

[P155]



A. Mouse IECs were treated with L-kyn or vehicle for 72 h. CYP1A1 mRNA was determined by real-time PCR. Treatment of endogenous ligand L-kyn induced AhR activation.  
 B. The cells were pre-treated with L-kyn alone or L-kyn + AhR antagonist CH-223191 for 6h and then stimulated with IL-1 $\beta$  for 24 h. Il-6 mRNA was determined. L-kyn abrogated IL-1 $\beta$ -mediated IL-6 expression through AhR pathway.

[P156]



## Graft-versus-host disease—clinical

relating with acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT), protecting endothelial cells (ECs) from damage may be a potent prophylaxis and therapeutic strategy of acute GVHD (aGVHD). Conventional aGVHD therapies may cause many adverse side effects because of their multiple targets. Therefore, we explored the therapeutic efficacy of simvastatin, a lipid-lowering drug, which has been demonstrated endothelial protection. Our previous clinical observation has found patients with aGVHD had lower Angiotensin-1 (Ang-1) level at day 7 but higher Ang-2 level at day 21 than those without aGVHD. In this study, we explored changes in Ang-1 and Ang-2 expression in an aGVHD mouse model and determined whether simvastatin prevents GVHD through regulating Ang-1 and Ang-2 expression. We preincubated EA.hy926 ECs with simvastatin (1mmol/l) 12 h before stimulated with TNF- $\alpha$ , then Ang-1 and Ang-2 concentration in the cell supernatant was measured by ELISA. Ang-1 and Ang-2 mRNA and protein level of treated and untreated cells were examined simultaneously. *In vitro* simvastatin increased Ang-1 production and release but conversely inhibited Ang-2 release from EA.hy926 ECs. Donor mice spleen cells were injected along with bone marrow cells into recipient mice after lethal irradiation to induce aGVHD. Simvastatin was administered orally once daily to mice (10 mg/kg) for 7 days after allo-HSCT and started -1 day after allo-HSCT. Then mice survival time was monitored and organ damage was evaluated. The plasma level of Ang-1 and Ang-2 was measured by ELISA, expressions of Ang-1 and Ang-2 in aortic endothelium were assessed by immunohistochemistry. Simvastatin improved the survival and attenuated the histopathological GVHD grades of aGVHD mice. Plasma levels of Ang-1 were significantly decreased, while plasma levels of Ang-2 obviously increased in aGVHD mice after transplantation. Simvastatin reduced plasma levels of Ang-2, elevated the plasma levels of Ang-1 as well as the aortic endothelial levels of Ang-1 and Ang-2. In summary, simvastatin represents a novel approach to combat GVHD by increasing Ang-1 production while suppressing Ang-2 release to stabilize endothelial cells.

**Disclosure of conflict of interest:** None.

### P157

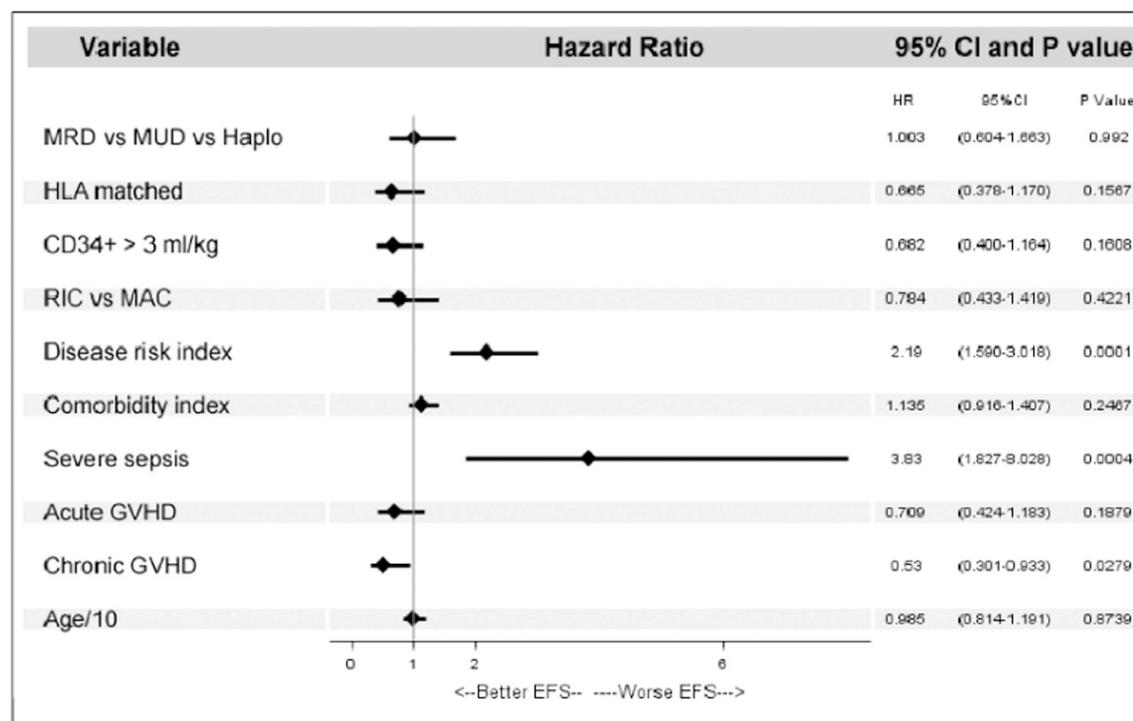
#### Two-year results of prospective trial of risk-adapted graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide in related, unrelated and haploidentical stem cell transplantations

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<sup>1</sup>R.M. Gorbacheva Memorial Institute of Hematology, Oncology and Transplantation, Pavlov First Saint Petersburg State Medical University

There is a growing evidence of safety and efficacy of post-transplantation cyclophosphamide (PTCy) in stem cell transplantations (SCT) from different donors and graft sources. Still the optimal combination of immunosuppressive agents with PTCy should be elucidated for different types of SCTs. We report the 2-year update of the prospective NCT02294552 single-center trial that evaluated risk-adapted graft-versus-host disease (GVHD) prophylaxis with PTCy in related, unrelated and haploidentical SCTs. 200 adult patients (median age 32 y.o., range: 18–62) with hematologic malignancies, including AML (47.5%), ALL (26.5%), CML (10.5%), MDS (4%), and lymphomas (11.5%), were enrolled in the study. 23% of patients were classified as salvage. 26% received the graft from matched related (MRD), 65% from matched/mismatched unrelated (MUD/MMUD), and 9% from haploidentical (haplo) donor. 43% received bone marrow graft (BM) and 57%-peripheral blood stem cell (PBSC) graft. 18.5% had myeloablative conditioning and 81.5%—reduced-intensity conditioning. GVHD prophylaxis for matched BM grafts consisted of single-agent PTCy 50 mg/kg days+3,+4, for matched PBSC graft—PTCy+ tacrolimus+ mycophenolate mofetil (MMF) 30 mg/kg days 5–35, and for any mismatched graft—PTCy+ tacrolimus+ MMF 45 mg/kg days 5–35. Median follow-up was 20 months (range: 4–40). Grade II–IV (10% vs 18% vs 11%,  $P=0.37$ ) and grade III–IV acute GVHD (4% vs 6% vs 0%,

[P157]



$P=0.59$ ) were not different in MRD, MUD/MMUD and haplo groups, respectively. Moderate and severe chronic GVHD was infrequent in all groups with slightly lower incidence after MUD/MMUD graft: and 22% vs 9% vs 21%,  $P=0.046$ . Non-relapse mortality (NRM) was not different after MRD, MUD/MMUD and haplo SCT (8% vs 14% vs 24%, respectively,  $P=0.19$ ), while relapse incidence was higher after MRD and haplo grafts: (45% vs 22% vs 52%,  $P=0.0017$ ). 2-year overall survival (OS), event-free-survival (EFS), and GVHD-relapse free survival (GFRS) were 73% vs 71% vs 44% ( $P=0.0015$ ); 48% vs 65% vs 33% ( $P=0.0008$ ); 29% vs 56% vs 22% ( $P=0.0002$ ) for MRD, MUD/MMUD and haplo groups, respectively. In the multivariate analysis only disease risk index (HR 2.2 95%CI 1.6–3.0,  $P=0.0001$ ), severe sepsis (HR 3.8 95%CI 1.8–8.0,  $P=0.0004$ ) and chronic GVHD (HR 0.53 95%CI 0.30–0.93,  $P=0.02$ ) were predictive for EFS, while type of donor was not a significant factor (HR 1.0 95%CI 0.6–1.7,  $P=0.99$ ) (Figure 1). The incidences of complications were: hemorrhagic cystitis—23%, sepsis—24%, severe sepsis—8%, invasive mycosis—8%, CMV reactivation—45%, veno-occlusive disease—2.5%, transplant-associated microangiopathy—3.5%, grade 3–4 liver toxicity—14%, grade 3–4 kidney toxicity—1%. More than one third of patients experienced poor graft function during 100 days after SCT, and in 83% of them CMV, HHV and BK virus reactivations were identified as the cause. The reported risk adapted strategy alleviates the risk of GVHD and NRM after MMUD and haplo grafts. The observed differences in the relapse incidence, OS and EFS were predominantly due to unbalanced disease risks in the groups. The relapse of underlying malignancy with this prophylaxis still significantly influences the outcome. Substantial number of patients experience poor graft function, which doesn't translate into NRM.

**Disclosure of conflict of interest:** None.

#### P158

##### **A high migratory capacity of donor T-cells in response to the lymph node homing receptor CCR7 increases the incidence and severity of graft-versus-host disease**

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Graft-versus-host disease (GvHD) pathogenesis involves migration of the donor T-cells into the secondary lymphoid organs (SLO) in the recipient, which is steered by two homing molecules: CD62L and CCR7. Therefore we investigated whether the migratory capacity of donor T-cells is associated with GvHD. This single center prospective study included 85 donor-recipient pairs. *In vitro* chemotaxis assays of the lymphocytes of the apheresis product were performed in parallel with the analysis of CD62L and CCR7 by flow cytometry. The potential of activation of CCR7+ T-cells was assessed through *ex vivo* activation assays with peripheral blood monuclear cells (PBMC) from healthy donors using anti-CD3 and anti-CD28mAbs. The migratory index to the CCR7 ligands, CCL19 and CCL21, was higher in T-cells from donors whose recipients will develop GvHD. These data indicated that the migratory capacity of the donor T-cells is clearly related to the development of GvHD. This prompted us to study the relationship between GvHD and the expression of two of the most relevant molecules in the trafficking of lymphocytes towards SLO, CD62L and CCR7, as a subrogate index of the migratory potential of T-cells. Consequently, we quantified the numbers of CD62L+ and CCR7+ T-cells in the graft. The initial transversal analysis of our data revealed that the percentage of CD62L+ lymphocytes in the apheresis product was very low compared to healthy lymphocytes. The analysis also confirmed that CD62L undergoes plasma membrane shedding after G-CSF mobilization thus making it a non-valid biomarker. The

analysis of CCR7 molecule revealed that the acute GvHD group received higher percentage of CD4+CCR7+ T-cells, whereas chronic GvHD patients were transplanted with higher percentage of CD8+CCR7+ T-cells compared to the non GvHD group. These results were confirmed when patients were subdivided into degrees of severity. A multivariate analysis was performed to investigate the real value of CCR7 to predict the development and severity of GvHD, and confirmed that CCR7 expression is a risk factor for the development of GvHD. Thus, the percentage of CCR7+CD4+ T-cells increases the probability of developing acute GvHD (OR=1.08, C.I (95%)=1.01–1.16,  $P=0.019$ ) and suffering a higher degree (OR=1.08, C.I (95%)=1.01–1.15,  $P=0.014$ ). Similarly, the OR of the percentage of CCR7+CD8+ T-cells was 1.17 (C.I (95%)=1.01–1.36,  $P=0.0031$ ) and 1.21 (C.I (95%)=1.05–1.39,  $P=0.006$ ) for the development of chronic GvHD and its degrees, respectively. Finally, to study the potential of activation of CCR7+ T-cells, we carried out *ex vivo* activation assays with PBMC from healthy donors using anti-CD3 and anti-CD28mAbs and the expression of CD40L on CD4+ T-cells and of CD69 on CD8+ T-cells as markers of activation, demonstrating that CCR7+ T-cells exhibited higher potential of activation than CCR7- T-cells. To our knowledge this is the first analysis of the influence of the migratory capacity of the donor T-cells on clinical outcome following allogeneic HSCT. Our data show that CCR7 could be considered a subrogate biomarker of the migratory capacity of the donor lymphocytes for predicting the risk of suffering GvHD. Based on the previous findings, we propose that the selective depletion of CCR7 expressing cells could be an effective preventive therapy for GvHD.

**Disclosure of conflict of interest:** None.

#### P159

##### **Previously published**

#### P160

##### **A single center research for outcome in patients receiving imatinib for steroid-refractory chronic GVHD after allogeneic stem cell transplantation**

L Ni, Y Luo, Y Tan, Y Hu, Y Zhao, J Shi and H Huang

Despite of major progress in allogeneic stem cell transplantation over the last decades, steroid-refractory chronic graft-versus-host disease (SR-cGVHD) remains a leading cause of late morbidity and mortality. Pre-clinical evidence confirms cGVHD has antibodies activating the platelet-derived growth factor receptor (PDGF-R) pathway. Since this pathway can be inhibited by imatinib, we performed a study including 16 patients with SR- cGVHD given imatinib at a dose of 300 mg per day. All patients with a median age of 25 years (range: 16–53) underwent allogeneic hematopoietic stem cell transplantation in our single center between 2008 and 2015, and chronic GVHD occurred at a median time of 10 months (range: 3–29) after transplantation. Patients had active cGVHD with measurable involvement of skin, lung or other districts and had previously failed in first-line immunosuppressive therapy. The major organs involved were lung ( $n=11$ ), skin ( $n=10$ ) and mouth ( $n=1$ ), including 5 cases involving both lung and skin, 8 cases involving 3 or more organs. According to the 2014 National Institutes of Health (NIH) criteria and NIH global severity, 13 patients were evaluated as severe cGVHD, and the other three were moderate. Meanwhile, the 2014 NIH Working Group had updated its recommendations for overall responses, consisting of complete remission (CR), partial remission (PR), and lack of response (unchanged, mixed response, progression). CR was defined as resolution of all manifestations in each organ or site, and PR was defined as improvement in at least 1 organ or site without progression in any other organ. After 3 months treatment, 14 patients receiving sufficient dose of imatinib revealed overall response rate (ORR) at 78.6%, and ORR remained unchanged at

6 months assessment, but with CR rate increased to 28.6%. Two patients couldn't meet the response of CR or PR were considered as a lack of response, including one evaluated as unchanged and one mixed response because of PR in lung accompanied by progression in eyes. With a median follow-up of 9 months, 14 patients were alive, with a 1 year estimated overall survival was 87.1%. 2 patients eventually died of pneumonia. Except 1 patient discontinued Imatinib because of grade 2 toxicity as gastrointestinal discomfort at the first month, no one had Imatinib-related grade 3 to 4 toxicity. This study suggests that imatinib is a promising and better tolerated treatment for patients with SR-cGVHD.

**Disclosure of conflict of interest:** None.

**P161**

**Analysis of REG3α as biomarker of gastrointestinal acute graft-versus-host-disease after nonmyeloablative HLA-haploidentical SCT with high-dose post-transplantation cyclophosphamide**

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Acute graft-versus-host-disease (aGVHD) is a major complication after allogeneic hematopoietic transplantation (allo-SCT). In recent years, a number of tissue-specific proteins have been described as biomarkers that could contribute to anticipate

and/or diagnose this complication earlier and more accurately. REG3α (Regenerating-Islet-Derived-3-alpha) has been directly related to gastrointestinal (GI) aGVHD. Our objective was to analyze plasma levels of REG3α at days +15 and +30 in patients who underwent unmanipulated haploidentical transplantation with reduced conditioning regimen (HAPLO-RIC), and to correlate the results with the development of aGVHD. We retrospectively analyzed 63 consecutive patients (2009–2016) who underwent HAPLO-RIC with post-transplant cyclophosphamide (days +3, +4), MMF and CsA as GVHD prophylaxis. Seven cases were excluded due to early death (before day +30) and 4 cases due lack plasma sample. Characteristics of the 52 patients included in the analysis are described in Table 1. REG3α detection was performed by ELISA (MBL International Corp, Woburn, MA) according to manufacturer's instructions on 200 μL of plasma obtained at day +15 and +30. The association of the incidence of aGVHD with known clinical variables and plasma REG3α levels were performed by Cox regression and Mann–Whitney *U*-test, respectively. The determination of the best cut-off of REG3α levels to stratify patients with GI aGVHD was performed with ROC curves. The statistical program used was R v2.15.0. The cumulative incidence of grade II–IV and grade III–IV aGVHD was 52% and 17%, respectively. Characteristics of aGVHD are shown in Table 2. No association was found between aGVHD and usual clinical variables (stem cells source, age, sex, conditioning regimen, donor/recipient sex and number of infused CD34+ cells), and with plasma REG3α levels at day +15. Plasma REG3α levels at day +30 were higher in patients who developed GI aGVHD compared to patients who did not showed GI aGVHD (median

[P161]

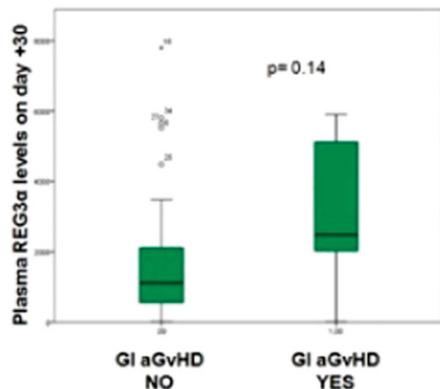


Figure 1. Plasma levels at day +30 based on GI aGVHD.

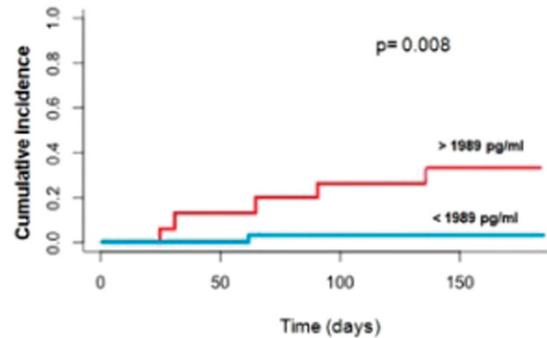


Figure 2. Cumulative incidence of GI aGVHD according to REG3α plasma levels at day +30.

Table 1. Patients characteristics	
Total, n	52
Sex, female/male, n/n	10/42
Median age, yr (range)	40 (17-66)
Diagnosis, n (%)	
HL/NHL	20 (39) / 9(17)
AML/MDS	10 (19) / 4(8)
Others (MDS, ALL,MM, LCC, Aplasia)	9(17)
Source of stem cells BM/PB, n/n	10/42
Median follow up, months	16 (4-88)
2 years OS,%	53 (46-70)
2 years EFS,%	50.3 (42,7-57,9)
2 years transplant related mortality ,%	19 (13,1-24,9)

MM: multiple myeloma; HL: Hodgkin Lymphoma; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; NHL: non hodgkin lymphoma; CLL: chronic lymphocytic leukemia; BM: bone marrow; PB: peripheral blood; OS: Overall survival; EFS: event free survival

Table 2. aGVHD characteristics				
		Gastrointestinal	Skin	Live
EICRa n=29	Number of patients, n	9	28	
	Day, median (range)	41 (27-136)	43 (18-150)	46 (18-150)
	Grade, n			
	II	5	22	
	III-IV	4	6	

aGVHD: acute Graft-versus-host disease

and range: 2483 (2022–5904) vs 1110 (0–7797) pg/mL,  $P=0.14$ , Figure 1). The best cut-off selected on day +30 was 1989 pg/mL (S85%, E71%). Patients with levels higher than 1989 pg/mL at day +30 had a significantly higher incidence of GI aGVHD grade II–IV (HR 6.9,  $P=0.008$ , Figure 2). Plasma levels of REG3 $\alpha$  at day +30 after HAPLO-RIC correlated with the occurrence of GI aGVHD grade II–IV. Therefore, plasma levels of REG3 $\alpha$  could be used for the prediction and/or diagnosis of GI aGVHD.

**Disclosure of conflict of interest:** None.

#### P162

##### **Anti-fibrotic treatment with pirfenidone in patients with GvHD-associated bronchiolitis obliterans syndrome**

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Prognosis of lung GvHD remains poor due to progressive decrease of lung function and repeated infections. Pirfenidone exhibits anti-fibrotic effects and has been shown to reduce disease progression in patients with idiopathic pulmonary fibrosis. Five patients with established BOS (NIH criteria 2014) and stable or deteriorating lung function under standard immunosuppressive treatment without active infection were treated with pirfenidone (2403 mg/d) in addition to their current therapy. Clinical assessments and pulmonary function tests were performed every three months. Five patients (4m, 1f), median age 60y (range: 29–65y) that were diagnosed with BOS at a median time of 13.5 months post-transplant started pirfenidone at a median time of 51 months (8–102) after diagnosis of BOS. Two patients are currently still under treatment after 611 and 638 days. Two patients had to stop treatment due to financial reasons after 189 and 206 days of therapy. One patient never reached more than 20% of the planned dose due to gastro-intestinal symptoms and was excluded from further analysis. At the start of treatment median FEV1 was 0.94L (0.72–1.34); 34.5% predicted (range: 21–44%) and median FVC 2.59 L (1.62–3.24); 46–84 % predicted. Median FEV1 trajectory was  $-0.65$  % predicted/month during median 6 months before start of pirfenidone (median  $-19$  mL/month) and  $+0.33$  % predicted/month ( $+9.8$  mL/month) during treatment with pirfenidone. The treatment was well tolerated except in one patient with gastro-intestinal complaints, no phototoxic reactions or serious drug-related adverse events occurred. In our small number of patients pirfenidone was rather well tolerated and generally safe. The observed, albeit small trend in change of FEV1 trajectory justifies further studies of anti-fibrotic therapy as a new therapeutic option in BOS after allogeneic HSCT.

**Disclosure of conflict of interest:** None.

#### P163

##### **Anti-thymocyte globulin (ATG) dose of 4.5 mg/kg vs 7.5 mg/kg did not result in a significant difference in the incidence of moderate to severe chronic graft vs host disease in allogeneic hematopoietic stem cell transplantation (HSCT) from unrelated donor**

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Anti-thymocyte globulin has been widely used for the prevention of severe graft versus host disease in patients undergoing HSCT from unrelated donor. However, the optimal dose remains to be defined. In Samsung Medical Center (Seoul, Korea), institutional strategy for the ATG use has been changed since April 2013, and we hypothesized that the incidence of chronic GVHD may differ by ATG strategy. Before April 2013, ATG 4.5 mg/kg was routinely used in allogeneic HSCT from unrelated donor, whereas, the dose of ATG was escalated to 7.5 mg/kg since April 2013. In this study, a total of

170 patients who underwent allogeneic HSCT from matched or unmatched unrelated donor between Jan 2010 and Dec 2015 were retrospectively analyzed. Peripheral blood was used as the source of stem cells in all patients. After a median follow up of 30.9 months, the cumulative incidence of moderate to severe chronic GVHD was 30.2% (95% Confidential interval [CI], 18.3 to 43.0) in the low-ATG group and 23.7% (95% CI, 14.5 to 34.1) in the non-ATG group ( $P=0.655$ ). The rate of 2-year overall survival (OS) was not significantly different between the groups (49.3% in low-ATG group vs 48.1% in high-ATG group,  $P=0.841$ ), as was the rate of disease free survival (DFS) (40.8% in non-ATG group vs 42.3% in ATG group,  $P=0.867$ ) and cumulative incidence of relapse (CIR) (23.9% in non-ATG group vs 24.5% in ATG group,  $P=0.776$ ). In allogeneic HSCT from unrelated donor, larger ATG dose (7.5 mg/kg) did not reduce the incidence of chronic GVHD when compared to lower ATG dose (4.5 mg/kg).

**Disclosure of conflict of interest:** None.

#### P164

##### **Anti-thymocyte globulin (ATG) significantly reduces the incidence of moderate to severe chronic graft versus host disease in allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling donor**

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Allogeneic HSCT provides a curative chance for patients with hematological fatal disease. However, substantial risks remain for morbidity and mortality caused by disease relapse and graft-versus-host disease. In Samsung Medical Center (Seoul, Korea), institutional strategy for the ATG use has been changed since April 2013, and we hypothesized that the incidence of chronic GVHD may differ by ATG strategy. Before April 2013, ATG was not routinely used in matched sibling donor (MSD) transplantation, whereas, ATG 5 mg/kg has been incorporated into HSCT process in transplantation from MSD thereafter. In this study, a total of 182 patients who underwent allogeneic HSCT from MSD between Jan 2010 and Dec 2015 were retrospectively analyzed. Peripheral blood was used as the source of stem cells in all patients. After a median follow up of 40.5 months, the cumulative incidence of moderate to severe chronic GVHD was 22.0% (95% Confidential interval [CI], 13.5 to 31.8) in the ATG group and 55.2% (95% CI, 42.9 to 65.8) in the non-ATG group ( $P=0.0018$ ). The rate of 2-year overall survival (OS) was not significantly different between the groups (62.5% in non-ATG group vs 58.7% in ATG group,  $P=0.624$ ), as was the rate of disease free survival (DFS) (61.1% in non-ATG group vs 53.3% in ATG group,  $P=0.377$ ) and cumulative incidence of relapse (CIR) (23.4% in non-ATG group vs 28.3% in ATG group,  $P=0.463$ ). In allogeneic HSCT from MSD, ATG use was significantly associated with less occurrence of chronic GVHD, but not linked to increasing risk of relapse, with showing similar OS and DFS between ATG and non-ATG group.

**Disclosure of conflict of interest:** None.

#### P165

##### **ATOS: A prospective multicenter non interventional observational study on the use of anti-human T-lymphocyte immunoglobulin (ATLG) in unrelated donor transplantation in adults with haematological malignancies**

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Long-term follow-up from the prospective randomized phase III multicenter trial comparing a standard GvHD prophylaxis with cyclosporine A and methotrexate with or without additional pretransplant ATLG (Grafalon, previously ATG-FRESENIUS S) (given 20 mg/kg/day, days -3 to -1) in unrelated donor hematopoietic cell transplantation after myeloablative conditioning resulted in a significant reduction of acute and chronic GvHD without compromising relapse rate and survival [1, 2, 3]. Here we report on a subsequent prospective non interventional observational study evaluating the outcome of patients receiving ATLG in unrelated donor transplantation in day to day clinical practice without the selective measures of a clinical trial (German clinical trials register DRKS00004581). Thirteen transplant centers included 165 patients with haematological malignancies (median age 54 years, IQR 45–61 years, range: 18–77 years) in early ( $N=75$ , 45%), intermediate ( $N=29$ ; 18%) or advanced ( $N=61$ ; 37%) disease status receiving marrow ( $N=6$ ) or PBSC ( $N=159$ ) from 10/10 matched (128; 78%) or mismatched (37; 22%) unrelated donors ( $N=4$  related) after myeloablative ( $N=100$ , 61%) or RIC ( $N=65$ , 39%) conditioning. GvHD prophylaxis consisted of calcineurin inhibitors, mainly CSA ( $N=154$ , 93%) with MTX or MMF and ATLG. Different dosing regimens were allowed according to current practise of centers. Median total ATLG dose was 46 mg/kg (IQR 32–60 mg/kg, range: 15–91 mg/kg). Median follow-up was 12 months (range: 8–14 months). As compared to patients in our randomized phase III multicenter trial [1, 2, 3], patients in this study were older; advanced disease status, 10/10 match, PBSC transplantation were more frequent, and given median ATLG dose was lower. Acute and chronic GvHD, NRM, relapse risk, DFS and OS at one year were similar to the results obtained in our randomized trial: incidence of  $^{\circ}$ II–IV aGvHD: 27%, III–IV aGvHD: 13%; moderate/severe cGvHD: 24%; NRM: 17%; risk of relapse: 23%; relapse mortality: 10%; OS: 73%. The experience in day to day clinical practice confirms the results shown in our randomized trial, namely the GvHD protective effect of ATLG without compromising NRM or relapse rates.

**References**

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- 2. Socie et al. *Blood* 2011; **117**: 6375
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**Disclosure of conflict of interest:** Speakers Bureau; research grants Neovii, Novartis, Riemsers.

**P166**  
**Baseline calprotectin as a predictor for acute gastrointestinal graft versus host disease (GVHD)—a prospective study**

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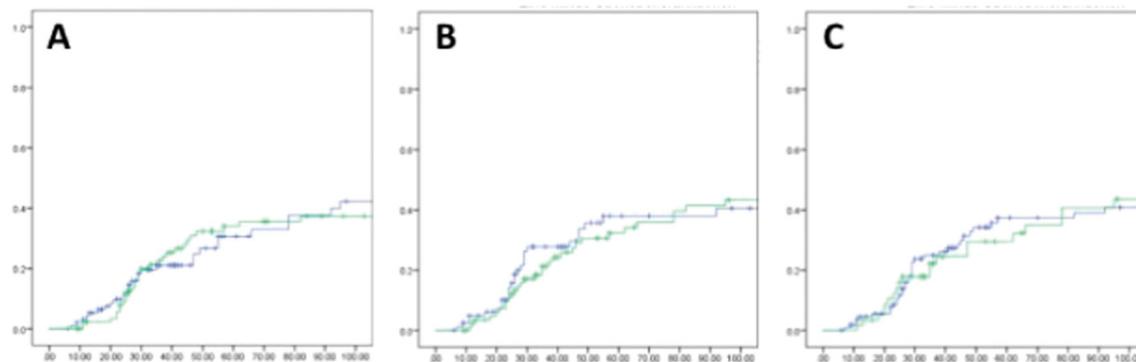
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Graft versus host disease (GVHD) is a major complication after allogeneic stem cell transplantation. So far there is no good validated predictor for the incidence and severity of GVHD. Fecal calprotectin (CPT) is a protein in leukocytes with antibacterial properties. It has been shown to be elevated in acute gastrointestinal GVHD. Additionally, CPT may be predictive for treatment response. The aim of the current prospective study was to investigate the role of baseline CPT in predicting incidence and severity of intestinal GVHD. In this prospective study conducted at the University Hospital Basel, Switzerland, we included all adult patients undergoing HSCT. The institutional review board approved the study. Data were collected prospectively. CPT was measured twice before conditioning and at transplantation. Fecal samples for CPT were obtained before conditioning and on the day of transplantation and assessed twice by standard ELISA. Between March 2012 and April 2016 a total of 194 patients (55% males, 154 patients with both baseline and transplant CPT values) were included. Patient, disease and transplant characteristics are described in Table 1. Median age at transplant was 55 years (range: 21–73 years). Most patients had myeloid neoplasia and 46% received myeloablative conditioning. GVHD prophylaxis consisted mainly of cyclosporine containing regimens (98%). CPT levels ranged from 19 to 1500  $\mu\text{g/g}$  both at baseline (median: 100  $\mu\text{g/g}$ ) and at transplantation (median: 101  $\mu\text{g/g}$ ), with a good consistency between the two measurements performed (internal quality control). On the other hand, CPT did not correlate with C-reactive protein. The two measurements were taken in median 7 days apart, depending on the conditioning regimen. Eighty-five patients had an increase of at least 50  $\mu\text{g/g}$  between baseline and transplantation. Overall 61 (31.4%) patients developed acute intestinal GVHD (grade 1: 19; grade 2: 18; grade 3: 16, and grade 4: 8 patients, respectively). CPT both at baseline and at transplantation was not predictive for the incidence of GVHD, acute intestinal GVHD, and for acute

[P166]

**Figure.** Incidence of intestinal GVHD until day 100 according to CPT at baseline (Figure A), at transplantation (Figure B) and for patients with an increase of CPT of at least 50  $\mu\text{g/g}$  between baseline and transplantation (Figure C).

Figure A & B: blue: CPT < 100  $\mu\text{g/g}$ ; green: CPT > 100  $\mu\text{g/g}$   
Figure C: blue: increase < 50  $\mu\text{g/g}$ ; green: increase > 50  $\mu\text{g/g}$



intestinal GVHD grade 3–4 (Figure). Additionally, we did not find a significant association between CPT levels and the above mentioned endpoints for patients showing an increase of CPT of at least 50 µg/g between baseline and transplantation. In the current prospective study, we didn't find any correlation between baseline CPT values and the incidence and severity of GVHD and intestinal GVHD. Further studies identifying early markers and predictors of GVHD are urgently needed.

[P166]

**Table 1. Patient, disease and transplant characteristics**

	Numbers (%)
Gender:	
- Male	106 (54.6%)
- Female	88 (45.4%)
Diseases:	
- AML	80 (41.2%)
- ALL	16 (.8.2%)
- PCN	24 (12.8%)
- B-NHL	21 (10.8%)
- MPN	13 (6.7%)
- MDS	20 (10.3%)
- T-NHL	11 (5.6%)
- MDS/MPN	6 (3%)
- SAA	3 (1.5%)
Donor:	
- Related	93 (47.9%)
- Unrelated	101 (52.1%)
Graft source:	
- PBSC	181 (93.2%)
- BM	12 (6.1%)
- CB	1 (0.5%)
CMV (Donor/Recipient):	
-/-	53 (27.3%)
-/+	60 (30.9%)
+/-	22 (11.3%)
+/+	59 (30.4%)
Blood group barrier:	
- None	111 (57.2%)
- Minor	38 (19.6%)
- Major	33 (17.0%)
- Bidirectional	12 (6.2%)
Conditioning:	
- Myeloablative	89 (45.9%)
- Non myeloablative	105 (54.1%)

**Disclosure of conflict of interest:** None.

## P167

### Calcineurin inhibitor (CI) free graft-versus-host disease (GvHD) prophylaxis: Its effects on resource utilization, renal function, and the cost of care

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Effective GvHD prevention following allogeneic hematopoietic stem cell transplantation (AH SCT) is vital to reducing transplant morbidity and mortality and improving overall outcomes. Several strategies are currently utilized for GvHD prophylaxis including MTX, MMF, CIs, post-transplant cyclophosphamide (Cy), and proteasome inhibitors. Recently, we described the results of a phase I-II trial of cyclophosphamide (Cy) and bortezomib (Bor) where patients (pts) received Cy (50 mg/kg) on days (d) +3 & +4 and Bor on d 0 & +3. The incidences of grade II-IV and grade III-IV acute GvHD were 35% and 11%. The incidence of chronic GvHD was 28%. In addition to GvHD, there are other factors that affect patients' quality of life and cost of care and that should be considered. It is well documented that CIs have an unfavorable toxicity profile. This includes nephrotoxicity and electrolyte disturbances. Furthermore, the CIs need serial level monitoring. Thus, we endeavored to compare the effects of CyBor combination against CI-based regimens by focusing on electrolyte requirements, specifically Mg, and renal function. We also sought to better understand financial considerations surrounding the need for CI drug level monitoring. Sixteen pts were randomly selected from the CyBor group and 16 patients from an internal control group of patients who received MMF and cyclosporine or tacrolimus following reduced-intensity AH SCT. The groups were well matched in regards to age, sex, disease status, PAM score, and baseline renal function. On each pt, Mg results from d 0 to +90 were compiled. Based on institutional protocol, a Mg replacement value was assigned as well as the corresponding drug and infusion charges. Next, the number of immunosuppressant (IS) trough levels from d 0 to +90 was tallied and the internal lab charges calculated. To compare renal function, GFR was calculated at baseline, d 0, and d +30.  $\chi^2$  tests and Wilcoxon Rank Square tests were used to analyze the data. For the CyBor group, median Mg value was 1.9 mg/dL (IQR 0.3) vs 1.7 (0.3) in the control group ( $P < 0.0001$ ). CyBor pts required a median of 7 grams (16) vs 49 grams (55) in the control group ( $P = 0.001$ ). The cost of Mg replacement and infusion was significant ( $P = 0.001$ ) (Table 1). For IS checks, drug levels were checked a mean of 0.625 times per patient in the CyBor group compared to 18.75 times in the control group ( $P < 0.0001$ ), which also translates to significant savings (Table 2). Considering these costs, the CyBor group saved ~\$6000. For GFR, 3 CyBor pts and 1 control pt had reduced GFR at baseline. On d +30, CyBor pts had better renal function in comparison to the control group ( $P = 0.018$ ) (Figure 1). In summary, CyBor significantly reduced the use of resources post-transplant and thereby the associated cost related to Mg replacement and need for drug level monitoring. Furthermore, CyBor preserved renal function at d +30. These findings could also impact patient's quality of life. Although our cost analysis was restricted to certain aspects of care and did not take into account other factors, it highlights specific important benefits of CI-free GvHD prophylaxis and supplicates further study. A formal prospective comparison of cost and QOL is warranted.

**Disclosure of conflict of interest:** ASA-H: funding from Millennium Pharmaceuticals.

## P168

### Cannabinoids in the management of chronic GVHD—experience of a center

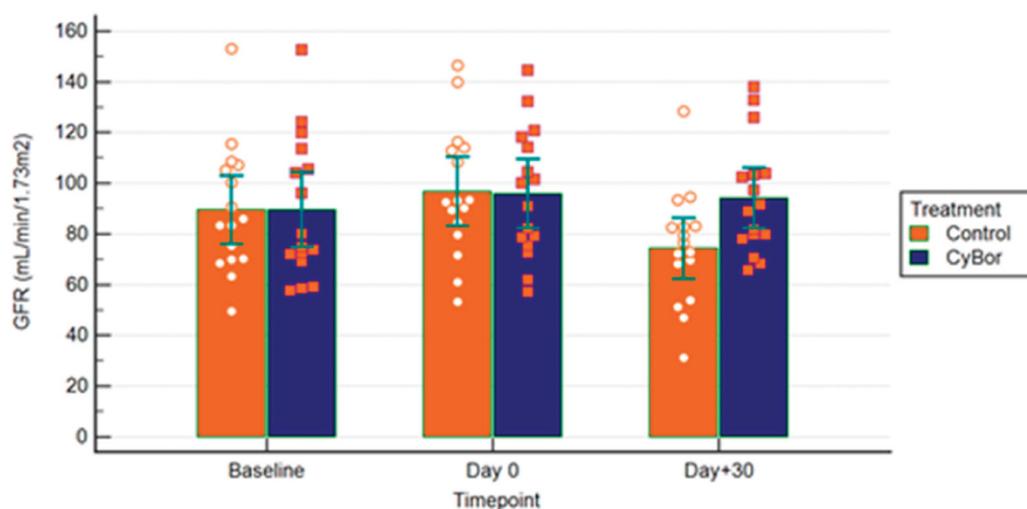
B Mesquita<sup>1</sup>, G Ferreira<sup>2</sup>, LL Corral<sup>3</sup>, D Riviera<sup>3</sup>, A Pita<sup>3</sup>, J Carrillo<sup>3</sup>, AR Guijo<sup>3</sup>, L Vázquez<sup>3</sup>, EP López<sup>3</sup>, M Cabrero<sup>3</sup>, D Caballero<sup>3</sup> and AA Martin<sup>3</sup>

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Table 1: Resource Utilization

Magnesium (Mg) Requirements and Costs by Median (Interquartile Range)	CyBor	Control	P Value
Mg Level mg/dL	1.9 (0.3)	1.7 (0.3)	<0.0001
Mg Infusions	3 (7)	16 (13)	0.001
Mg (gms) Given	7 (16)	49 (55)	0.001
Infusion Charges	\$482 (1116)	\$2924 (3288)	0.001
Mg Therapy Cost	\$456 (1043)	\$3194 (4042)	0.001
Immunosuppressant (IS) Cost by Mean (Range)	CyBor	Control	P Value
Number of IS Checks	0.625 (0-6)	18.75 (14-30)	<0.0001
Cost of IS Checks	\$30 (0-305)	\$866 (688.82-1412)	<0.0001

Figure 1: Renal Function by Immunosuppressant Type



The Graft versus host disease (GVHD) represents a therapeutic challenge in allotransplantation. The therapeutic potential of cannabinoids in the management of GVHD was first described by Pandey R. (2011), and in acute GVHD by Yeshurun M. (2015). The cannabinoids use in spastic symptoms of Multiple Sclerosis is reported by Patti F. (2016), and its paper in the fibrogenesis were reported by Li SS. (2016), raising the possibility of its therapeutic utility in sclerodermal symptoms and stiffness in the context of chronic GVHD (cGVHD). Characterize a group of patients with cGVHD treated with Sativex (oral spray with THC and Cannabidiol). Retrospective analysis of patients with cGVHD treated with Sativex between 02/10/2015 and 09/01/2016. 16 patients were treated in our center (10 began in 2016) with a sex ratio 1:1. The median follow-up for patients alive was 213 days (76–561). Initial diagnosis: ALL/lymphoblastic lymphoma  $n=3$ , AML  $n=4$ , CML  $n=2$ , Myelofibrosis  $n=1$ , HL  $n=1$ , NHL  $n=2$ , MDS  $n=3$ . Transplanted between 2005–2015, with median age of 53 years (16–67). Related Donor  $n=11$  and Unrelated Donor  $n=5$  (compatibility 9/10  $n=1$ ), with conditioning regimen: Myeloablative  $n=5$  and Non-myeloablative  $n=11$ . Median interval

between transplantation and diagnosis of cGVHD of 11 months(5.1–40.2); and between cGVHD diagnosis and Sativex 23 months (9.1–105.3), with a median of 4 prior treatment lines (2–9). At the time of beginning, the cGVHD was extensive in all patients, severe cGVHD  $n=5$  and moderate cGVHD  $n=11$ . All patients except one had cutaneous involvement ( $n=13$  with sclerodermal features). In addition, other organs were affected: digestive  $n=2$ , pulmonary  $n=7$ , hepatic  $n=4$ , ocular  $n=8$ , oral  $n=9$ , genital  $n=2$  and muscular  $n=7$ . Drug was started because of pulmonary affectation in 3 patients and due to sclerodermal/muscular involvement in 13 patients. Concomitant therapies during treatment were: topical cutaneous treatment  $n=11$ , topical ocular treatment  $n=10$ , pulmonary  $n=7$ , sirolimus  $n=8$ , tacrolimus  $n=3$ , oral corticosteroids  $n=9$ , extracorporeal photopheresis  $n=4$ , ruxolitinib  $n=2$ , imatinib  $n=1$ , mesenchymal stem cells  $n=1$ . The mean dose were three puff/day (2–5), with good tolerance, only two discontinuations of treatment because of adverse effects. Median time of treatment 156 days (45 to 561). At the time of the analysis 11 patients were still under treatment. Responses mainly occurred within the first 60 days,

with a median time of duration of 106 days (20 in 450). Responses after two months of treatment were: 6 partial organ response, 4 mixed responses, 4 unchanged and 2 organ progressions; at 120th day (14/16) only two patients maintained their responses (one PR and one mixed response). It must be pointed out that one patient who reached PR with sativex in monotherapy maintain response after 18 months of treatment. In addition, cramps were resolved in 5 patients. Sativex appears to be an effective treatment option in patients with chronic GVHD, particularly in those having cramps, sclerodermal features and pulmonary affection. As seen in Multiple Sclerosis context, the main issue with its use is the loss of response in the long-term follow up. The median dose is inferior to the one described in MS, leaving the question if higher doses can deepen the response. These results should be confirmed in prospective trials.

**Disclosure of conflict of interest:** None.

#### P169

##### **Characterization of GVHD and its risk factors in patients undergoing allogeneic haematopoietic progenitor cell transplantation**

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Several risk factors associated with acute and chronic graft-versus-host-disease (GVHD) have been identified in multiple studies. Most commonly associated factors are human leukocyte antigen (HLA), mismatch between recipient and donor, as well as several other characteristics such as age, conditioning regimen and prior acute GVHD. **Objective:** The aim of this study was to evaluate the characteristics of acute and chronic GVHD in patients who underwent an allogeneic hematopoietic stem cell transplantation (HSCT), identify differences in the profile risk factors for acute and chronic GVHD and their impact in post-transplant morbidity and mortality. This retrospective study included 90 Mexican adult patients who received an allogeneic HSCT between January 2010 and March 2016, at Instituto Nacional de Cancerología. We analyzed 90 patients with a median age of 30 years (15–64), from which, 60% were male patients. Among the participants with hematologic malignancies, 39 were previously diagnosed with acute lymphoblastic leukemia, 20 acute myeloid leukemia, 11 chronic myeloid leukemia, 8 lymphoblastic lymphoma and 3 with myelodysplastic syndrome. Because bone marrow transplants are not performed at this Institution, all transplants were from peripheral blood stem cell harvest. Acute GVHD prophylaxis consisted in a triple immunosuppressive drug regimen for all patients. 86.7% of the patients had high risk disease prior to HSCT. Myeloablative conditioning represented 82% of the applied regimens, which consisted of IV busulfan in 63.3% of the cases. 44.9% of patients, were transplanted within 12 months from diagnosis. The cumulative incidence of acute GVHD at 100 days was 21.1% (19 patients). Patients with acute GVHD had 42% grade A, 15% grade B and 42% grade C, according to the IBMTR grading system. 12 patients had skin involvement, with grade 1–2 acute GVHD in 83% of the cases, 4 patients developed liver involvement and 6 patients had gastrointestinal tract disease. 19% of the patients developed chronic GVHD, from which, 57% were classified as severe, 10.5% as moderate and 21.6% as mild. 36% of the patients who developed chronic GVHD had a single organ involvement, while 26.3% had 3 or more organs/sites. Prior acute GVHD was associated with development of chronic GVHD. The multivariate analysis identified HLA unrelated donor as the only risk factor associated with the development of acute GVHD (HR, 5.1; 95% CI, 3.3–7.9,  $P=0.043$ ). The overall survival at 5 years was of 69% poor patients who developed acute GVHD and of 34% for

those who didn't ( $P=0.065$ ). Our analysis showed that the incidence of acute and chronic GVHD at our center is lower than the reported at other centers, but we were not able to identify risk factors usually associated with the development of GVHD, perhaps due to the small population that we evaluated.

#### **Reference**

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**Disclosure of conflict of interest:** None.

#### P170

##### **Previously published**

#### P171

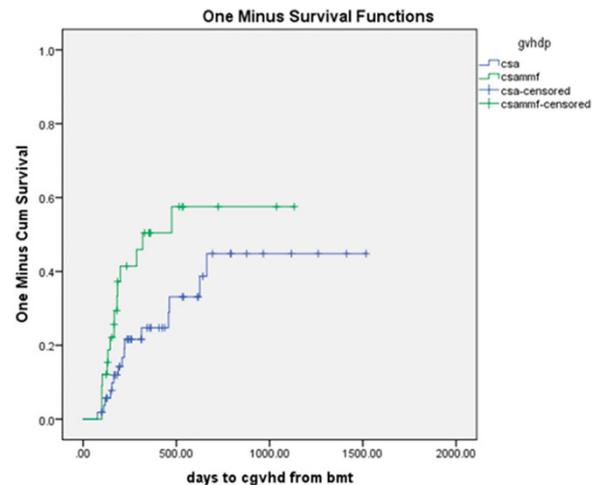
##### **Ciclosporin toxicity: Increased mortality and incidence of chronic GvHD after stopping Ciclosporin A and replacing it with mycophenolate mofetil as prophylaxis for GvHD**

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Graft versus host disease (GvHD) remains one of the main obstacles to broader application of allogeneic stem cell transplantation (SCT). Despite the routine use of prophylactic therapies, chronic GvHD (cGvHD) occurs in 10 to 80% of patients undergoing allogeneic SCT. Ciclosporin A (CsA) remains the backbone for GvHD prophylaxis in both myeloablative (MAC) and reduced intensity conditioning (RIC) SCT. However, in a significant proportion of patients, CsA causes important side effects and needs to be discontinued. In this study we have evaluated the impact of substituting CsA for Mycophenolate Mofetil (MMF) as immunosuppression (IS), on the incidence of cGvHD. We have compared the outcome of 87 consecutive patients that underwent allogeneic SCT from March 2011 to November 2015 at the BMT Unit of the Hammersmith Hospital and received CsA as part of the GVHD prophylaxis. Of them, 54 patients (62%) remained on CsA prophylaxis for the duration of the planned post SCT immunosuppression period and 33 patients (38%) required a switch to MMF before day +100. The reason for changing the IS was nephrotoxicity in the majority of cases ( $n=25$ , 70%), neurological toxicity ( $n=2$ , 6%), disease relapse ( $n=1$ , 3%), intolerance ( $n=2$ , 6%) or not determined ( $n=3$ , 9%). We excluded from the analysis those patients whose IS was changed due to the presence of acute GvHD. Both groups had similar patient and transplant characteristics (see table 1).

#### [P171]



However, distribution according to diagnosis showed a predominance of AML (43%) in patients that remained on CsA and MDS (2%) for those that switched to MMF. The mean survival rate of the entire cohort was 902.897 days ( $\pm 87$ ). The mean survival of each group was: CsA 996.86 days ( $\pm 109.076$ ) and MMF 602.474 ( $\pm 94.779$ ). This difference in survival reached statistical significance ( $P=0.04$ ). We graded cGVHD using the NIH scoring system as mild, moderate and severe. Out the 54 patients that continued with CsA, 55.6 % ( $n=30$ ) had no cGVHD; 16.7 % ( $n=9$ ) had mild cGVHD; 20.4 % ( $n=11$ ) had moderate and 7.4 % ( $n=4$ ) had severe cGVHD. In patients that switched to MMF 45.5 % ( $n=15$ ) did not develop any cGVHD; 9.1 % ( $n=3$ ) developed mild; 24.4 % ( $n=8$ ) moderate cGVHD and 21.25 % ( $n=7$ ) developed severe cGVHD. ( $P: 0.093$ ). the cumulative incidence of any CGVHD at 2 years post SCT was 58% for the CSA/MMF group and 42% for the CSA only group ( $P=0.04$ ). CsA is one of the standards of care for GVHD prophylaxis in both RIC and MAC SCT. In our cohort of patients, those who remained on CsA had a better overall survival and a reduced incidence of chronic GVHD compared with those patients that stopped CsA and replaced it by MMF. CsA toxicity should be prevented to avoid GVHD-related complications.

**Disclosure of conflict of interest:** None.

### P172

#### Clinical impact of different anti-thymocyte globulin doses as GVHD prophylaxis in patients receiving stem cell transplantation from unrelated donors

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Although the outcome of allogeneic stem cell transplantation (SCT) from an unrelated donor (UD) has considerably improved over the recent years, graft versus host disease (GVHD) still represents a severe and potentially lethal complication. *In vivo* T-cell depletion with anti-thymocyte globulin (ATG) has been shown to significantly decrease the risk of both acute and chronic GVHD without compromising survival, however the optimal dose has not been defined yet. Aim of present retrospective study was to evaluate the impact of two different doses of rabbit ATG (Thymoglobulin) on GVHD incidence, infectious complications and outcome of 156 patients undergoing SCT from UD. Between February 2004 and September 2015, 40 patients received Thymoglobulin 5 mg/kg (ATG-5 group) and 116 received Thymoglobulin 7 mg/kg (ATG-7 group) in addition to cyclosporin and short course MTX as GVHD prophylaxis. The two groups were comparable regarding sex, age, diagnosis and disease phase at transplant, comorbidity index, stem cell source and antimicrobial prophylaxis. Conditioning treatment was myeloablative in 90% of ATG-5 group patients and in 78% of ATG-7 group patients. Donor and recipient pairs were 10/10 HLA matched in 75% of the cases of the ATG-5 group and in 39% of the

cases of the ATG-7 group ( $P 0.001$ ). Neutrophil engraftment occurred in 150 (96%) patients at a median of 17 days post transplant (range: 11–41 days); six patients (2 in the ATG-5 group and 4 in the ATG-7 group) died before engraftment. Overall, 48 patients (31%) developed grade II–IV acute GVHD, without significant differences between the two groups (ATG-5 32% and ATG-7 30%,  $P 0.939$ ). Similarly, chronic GVHD was not significantly different between the two groups: moderate to severe chronic GVHD occurred in 30% of the patients in the ATG-5 group and in 27% of the patients in the ATG-7 group ( $P 0.846$ ). Univariate logistic regression analysis didn't show any significant differences between the two groups respect the incidence of bacteremia, invasive fungal infections acute and chronic GVHD. With a median follow-up of 66.6 months, 84 patients (54%) are alive, 79 in complete remission and 5 after disease relapse. Transplant related mortality was superimposable in the two groups (ATG-5 17% vs ATG-7 20%). Kaplan–Meier estimates of overall survival and event free survival were 54% and 52%, respectively, without statistically significant differences between the two groups and between HLA matched and HLA mismatched SCT. The results of our study suggest that different doses of ATG tailored on HLA compatibility might be effective for preventing GVHD with any detrimental effect on overall survival and incidence of infectious complications. A prospective randomized study is mandatory to confirm our preliminary results. **Disclosure of conflict of interest:** None.

### P173

#### C-reactive protein levels at acute GVHD diagnosis predict steroid-resistance, treatment related mortality and overall survival after allogeneic hematopoietic stem cell transplantation

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Acute graft versus host disease (aGVHD) remains an excessive cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Primary treatment consists of high-dose corticosteroids, but a small group of patients are steroid-resistant and their prognosis is especially poor. A predictor of patients at risk of steroid-failure would aid the decision of additional immunosuppressive treatment at an early stage. There is experimental evidence that co-existing inflammation aggravates aGVHD. Since C-reactive protein (CRP) is a systemic inflammatory marker, we aimed to investigate whether CRP levels at aGVHD diagnosis could predict the risk of failing first-line therapy and developing steroid-resistance. We retrospectively studied 461 patients transplanted between 2010 and 2015, table 1. Acute GVHD was diagnosed in 204 patients, 149 of whom had grade II–IV. CRP, total white blood cell-, lymphocyte- and neutrophil counts were available for all patients at the time of aGVHD diagnosis. According to local protocol, patients with failed response to high-dose steroid (2 mg/kg) were treated with the tumor necrosis factor (TNF) alpha inhibitor infliximab and categorized as steroid-resistant. Of 149 grade II–IV aGVHD patients 28 (19%) developed steroid resistant disease. CRP levels at diagnosis among these were between  $< 1$  and 263 mg/L. CRP levels were significantly higher in patients who developed steroid resistance compared to patients responding to high-dose corticosteroids,  $P=0.001$ , HR 1.011 (95% CI 1.005, 1.018). This translated into significantly increased transplant-related mortality (TRM) and decreased overall survival in patients with high CRP levels, Figure 1. Total white blood cell-, lymphocyte- and neutrophil counts were not associated with steroid resistance in aGVHD patients.

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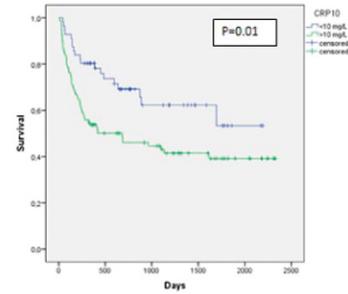
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<b>Table 1. Patient and transplant characteristics</b>	
Patients, n	461
Follow-up time, days median, range	1340 (311–2480)
Age, years median, range	53 (16–75)
Disease, n	
AML	173
MDS	111
ALL	58
NHL	37
CLL	27
CML	18
SAA	15
Other	22
Donor, n	
Sibling	117
Matched unrelated	344
Stem cell source, n	
Bone marrow	118
PBSC	343
Conditioning regimen, n	
Myeloablative	201
Nonmyeloablative	260
aGVHD, n	204
aGVHD II-IV, n	149

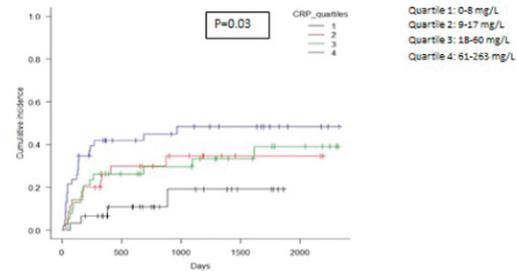
These results suggest CRP levels at diagnosis as a valid predictor of developing steroid resistant disease in aGVHD grade II-IV and survival in allogeneic hematopoietic transplant recipients.

**Figure 1**

1a. Kaplan-Meier estimate of overall survival by CRP levels above and below 10 mg/L at diagnosis in patients with grade II-IV aGVHD, n=149.



1b. Cumulative incidence of TRM with relapse as competing event by CRP quartiles at diagnosis in patients with grade II-IV aGVHD, n=149.



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**Disclosure of conflict of interest:** None.

**P174**

**CXCR3 autoantibodies and ligands in acute GVHD—bridging endothelial and T cell pathology**

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CXCR3 is chemokine receptor expressed on activated T lymphocytes, in particular on Th1 cells, NK cells, dendritic cells, and subsets of epithelial and endothelial cells. CXCR3 ligands attract Th1 cells into inflamed tissues and concomitantly block the migration of Th2 cells. Furthermore, inhibitory functional autoantibodies against CXCR3 occur in humans which play an important role in CXCR3-dependent immune regulation. In addition, CXCR3 regulates endothelial cell homeostasis. There are two variants of CXCR3: CXCR3-A and CXCR3-B. Overexpression of CXCR3-A on endothelial cells is associated with an increase in cell survival, whereas overexpression of CXCR3-B dramatically reduced DNA synthesis and up-regulated apoptotic endothelial death. Here we have studied if a dysfunctional CXCR3 axis might be involved in GVHD pathogenesis and could link endothelial and T cell pathology in acute GVHD. We assessed concentrations of the CXCR3 ligands CXCL9, CXCL10 and CXCL11 as well as anti-CXCR3 autoantibodies in 98 patients with high grade (3–4) acute intestinal GVHD for whom serum was available at GVHD onset. Furthermore, anti-CXCR3 autoantibodies and CXCL9 levels were measured in sera stored before conditioning therapy. All variables were tested for influence on post-GVHD survival using cause-specific Cox regression analysis. At GVHD onset, we observed a strong inter-correlation of CXCR3 ligands, but no correlation with anti-CXCR3 auto-antibodies. Compared with pre-conditioning probes, CXCL9 levels strongly increased (median 303 to 721 pg/mL,  $P < 0.001$ ), whereas anti-

CXCR3 decreased (median 4.4 to 2.6 U/mL,  $P < 0.001$ ). Anti-CXCR3 levels before conditioning and at GVHD onset correlated (coeff. 0.497,  $P < 0.001$ ), whereas CXCL9 levels did not. In multivariable analyses, low anti-CXCR3 and high CXCL9 measured at disease onset were strongest predictors of survival after acute GVHD. Notably, high levels of the pro-inflammatory chemokine CXCL9 were particularly prognostic of an adverse outcome of GVHD in the presence of a high endothelial risk as assessed by the previously published EASIX score, while high anti-CXCR3 levels were most protective in patients with low EASIX score (that is, low endothelial risk). A score based on CXCL9, anti-CXCR3, and EASIX allowed an effective prediction of acute GVHD outcome ranging from mortality  $>90\%$  (high CXCL9 + high EASIX) to mortality  $<20\%$  (low CXCL9, low EASIX, high anti-CXCR3). Our data suggest a strong role for the CXCR3 axis in the pathology of acute high grade GVHD. The opposing effects of CXCL9 and anti-CXCR3 indicate a functional, attenuating role for these auto-antibodies. The overall prognostic impact of the immune-modulating CXCR3 axis appears to depend on the underlying integrity of the patients' endothelial homeostasis.

**Disclosure of conflict of interest:** Intellectual property pending for analysis of anti-CXCR3: HH, TL.

### P175

#### Does pretransplant imatinib treatment influence chronic GvHD occurrence? Ph positive vs Ph negative acute lymphoblastic leukemia patients preliminary comparative analysis on behalf of polish adult leukemia group

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Despite some progress in acute lymphoblastic leukemia (ALL) treatment including modern chemotherapy modalities, monoclonal antibodies and newer tyrosine kinase inhibitors (TKI) for Ph positive cases, the final success is still difficult to reach. Allogeneic hematopoietic stem cells transplantation (alloHSCT) has remained an essential approach in attempts to cure ALL. TKI routinely used for ALL Ph(+) pre- and post-transplant treatment are also described as an alternative and adjunctive approach for chronic GvHD especially with fibrotic features due to their antifibrotic activity targeting the platelet-derived growth factor receptor (PDGFR) pathways. In this study we have tried to estimate the potential influence of pretransplant TKI treatment on GvHD occurrence comparing ALL Ph(+) and ALL Ph(-) cases treated with alloHSCT. A cohort of 119 ALL patients consisted of 93 ALL Ph(-) and 26 ALL Ph(+) cases treated with alloHSCT was retrospectively analyzed. All patients were transplanted from sibling or unrelated donor (no haploidentical procedures were included). All Ph(+)

patients achieved pretransplant treatment with Imatinib and chemotherapy, and Ph(-) patients with chemotherapy alone. The median age in Ph(-) and Ph(+) group was 28 vs 35 ( $P = 0.04$ ), the percentage of HLA mismatched transplantations—4.3 vs 19.2 ( $P = 0.00004$ ), the percentage of acute GvHD cases—48.4 vs 76.9 ( $P = 0.01$ ) and extensive chronic GvHD cases—80.5 vs 50.0, respectively. There were no significant difference between groups in patients sex (F/M—41/52 vs 14/12 respectively), RIC/MAC conditioning, unrelated/sibling donor, donors age, BM/PBPC transplantation, number of CD34 cells and chronic GvHD incidence. All patients received Cyclosporine- and Methotrexate-based GvHD prophylaxis. GvHD occurrence was analyzed in subgroups as previously described: ALL Ph(-) and ALL Ph(+). As mentioned above the incidence of acute GvHD was higher in Ph(+) group (higher number of HLA mismatched transplantations in this group) but the incidence of extensive chronic GvHD was higher in Ph(-) group. Cox proportional hazard model analysis revealed death risk caused by GvHD higher in Ph negative group (hazard ratio = 2.3; CI 95% = 1.02–5.18;  $P = 0.04$ ). The analysis of competing events was performed to estimate the probability of death caused by GvHD vs other complications (transplant related mortality, infections and relapse). The impact of conditioning was not significant on GvHD related deaths vs other complications ( $P = 0.234$  vs 0.009, respectively—Figure 1). The same results were achieved with donor CMV status ( $P = 0.09$  vs 0.04—Figure 2). We have not found any significant difference either in GvHD or other complications related deaths taking into account patient's sex/age, donor sex/age, patients CMV status, number of CD34 cells transplanted. On the other hand, the influence of aGvHD and chGvHD on deaths related to other complications was not significant ( $P = 0.242$  vs 0.147). Cumulative probability of overall survival was higher in Ph(+) group but the difference was not significant. The impact of pretransplant treatment with Imatinib on GvHD occurrence has not been estimated so far. We are aware of our results to be preliminary and variety of data is still to be evaluated. However our results, if confirmed, may suggest the influence of imatinib on decreasing the extensiveness of chronic GvHD.

**Disclosure of conflict of interest:** None.

### P176

#### Dose dependent association of early red blood cell transfusion with increased risk of graft versus host disease and worse survival after allogeneic hematopoietic stem cell transplantation

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Figure 1. Competing events – the influence of conditioning on GvHD and other complications related deaths

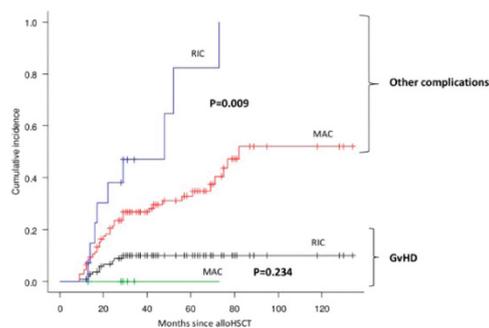
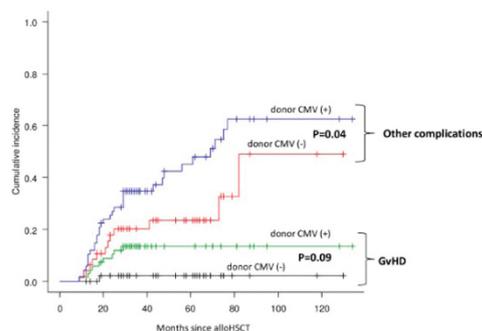


Figure 2. Competing events – the influence of donor CMV status on GvHD and other complications related deaths



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More than 90% of allogeneic hematopoietic stem cell transplant (allo-HSCT) patients receive red blood cell (RBC) and platelet (PLT) transfusions in the peritransplant period. Preclinical models indicate that RBC and PLT transfusions trigger inflammation, raising the question of whether such transfusions are associated with development of severe acute graft-versus-host disease (grade III/IV aGvHD) and mortality in

allo-HSCT recipients. We conducted a retrospective analysis of RBC and PLT transfusions, aGvHD incidence, and mortality among 322 consecutive adult patients receiving non-T cell-depleted allogeneic bone marrow (11%) or G-CSF-mobilized blood stem cell grafts (89%). Common underlying diseases were acute myeloblastic leukemia (41%), myelodysplastic syndrome (14%), and acute lymphoblastic leukemia (12%). Underlying disease risk was ranked as low (41%), intermediate (26%) or high (32%). Allografts were obtained from 10/10 HLA-matched sibling donors (35%), unrelated donors (43%), or from donors mismatched at 1–2 HLA alleles (22%). Graft sources were bone marrow (11%) or mobilized PBSC (89%). The cumulative incidences of grade III–IV aGvHD and mortality prior to day 150 without developing grade III/IV aGvHD were estimated using the cumulative incidence function and a Cox proportional hazards regression model. Covariates included in multivariable analysis was limited to baseline covariates associated with grade III/IV aGvHD at the P median number of RBC or PLT transfusions (Figure 1). Univariate analysis showed a lower hematocrit on admission (median of 5 RBC units transfused ( $P=0.001$ )) were significantly associated with the risk of developing grade III/IV aGvHD, while a longer time to neutrophil engraftment was inversely associated with grade III/IV aGvHD ( $\geq$  median of 15 days, HR 0.58,  $P=0.03$ ). Multivariate Cox regression analysis showed only larger numbers of RBC units transfused and HLA mismatch independently associated with severe aGvHD ( $P=0.02$  and  $P=0.008$ , respectively), while underlying disease risk and larger numbers of transfused RBC units were independently associated with overall survival in a multivariate analysis that excluded aGvHD grade. Overall mortality rate was lowest for the group with fewer RBC and PLT transfusions (43%), and greatest for the group with more RBC and PLT transfusions (67%). Groups that received more RBC units had higher rates of mortality due to GvHD, while patients who received more PLT transfusions and fewer RBC transfusions had greater mortality from relapse (Figure 2). These data support the hypothesis that peritransplant RBC transfusions are associated with the risk of developing severe aGvHD and worse overall survival following allo-HSCT. Prospective studies are warranted to whether RBC transfusions promote T-cell activation and inflammation in allo-HSCT recipients, leading to increased severe aGvHD.

**Disclosure of conflict of interest:** None.

**P177**

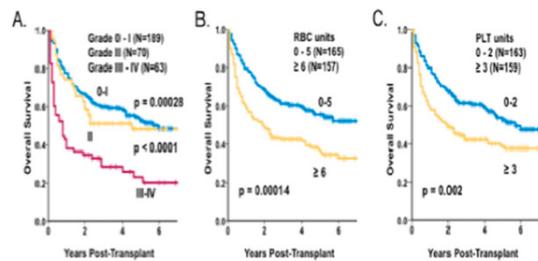
**Early high umbilical cord blood CD3 chimerism associated with acute GVHD at time of onset in haplo-cord transplantation**

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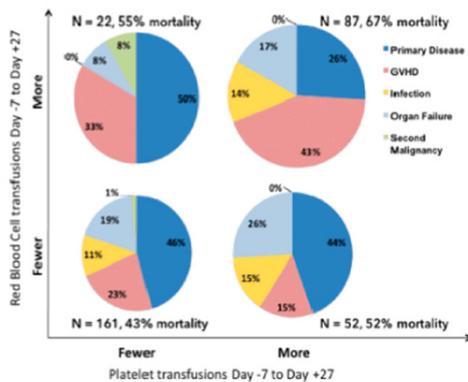
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**Introduction:** Haplo-cord transplantation is a combined haploidentical and cord blood transplant that allows for more rapid engraftment by the haplo with eventual loss of the haplo graft upon engraftment of the cord. Haplo-cord transplants are associated with an approximate 25–43% incidence of aGvHD. (1.2) However, at the time of onset of aGvHD, it is unclear which graft or if both are contributing. We have recently

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**Figure 1. Overall survival was associated with aGvHD grade and numbers of RBC units and platelet units transfused from day -7 to day +27 in Kaplan-Meier analyses.** A) Kaplan-Meier analyses of cumulative survival comparing patients with grade 0/I (blue), II (yellow) and III/IV (maroon). B) Cumulative survival of patients transfused with 0-5 RBC units (blue) vs. 6 or more RBC units (yellow). C) Cumulative survival of patients transfused with 0-2 platelet units (blue) vs. 3 or more platelet units (yellow). Plots are truncated at 7 years post-transplant.



**Figure 2. Causes of death in 4 patient groups based on RBC and platelet transfusions. Groups that received more RBC transfusions had higher frequencies of GvHD-related mortality.** Patients were grouped based upon the number of total RBC and platelet units received from day -7 to +27 post-transplant, including those who received after a diagnosis of aGvHD (0-6 vs.  $\geq 7$  RBC units, and 0-3 vs.  $\geq 4$  platelet units). Pie chart sizes are proportional to the % mortality in each group.

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		days to aGVHD/days to chimerism	CD3 recipient %	CD3 haplo %	CD3 cord %	CD33 recipient %	CD33 haplo %	CD33 cord %
aGVHD n=31	median	47.0	0.0	0.0	100.0	0.0	88.0	12.0
	mean	48.1	0.9	10.9	88.2	2.2	58.5	39.3
no aGVHD n=59	median	57	0	0	88	0	54	23
	mean	60.2	18.2	15.2	65.2	2.9	53.0	44.1

reported, using chimerism assessments at approximately day 56 after transplant for AML and MDS, that lower umbilical cord blood (UCB) chimerism in the CD3 or CD33 lineages were associated with increased rates of relapse. We did not find a statistically significant association between day 56 chimerism and risk for acute GVHD.(2) Here we report our analysis of chimerisms at the onset of aGVHD. Patients and Methods We retrospectively reviewed all patients who underwent haplo-cord SCT for all hematologic malignancies between July 2012 and March 2016. UCB for haplo-cord transplants were selected based on HLA-typing and cell count. Grafts were matched for at least 4 of 8 HLA loci by the standard criteria and contained a minimum cell count of  $1 \times 10^7$  nucleated cells per kilogram (kg) of the recipient's body weight before freezing. The haplo-identical donor was a relative in the large majority of cases. We identified 90 patients evaluable for aGVHD (onset before Day 100) without preceding relapse or early death. Of the total 90 patients, 31 patients were diagnosed with aGVHD of any stage and grade. Fractionated chimerisms including CD3 and CD33 components were routinely sent to evaluate for engraftment of the recipient vs haplo vs UCB. Chimerism data was collected for both aGVHD and no aGVHD patients. The two-sided Student's t-test was used to compare the aGVHD cohort to the no aGVHD cohort. Chimerisms collected on patients with aGVHD were within median  $\pm 4$  days of onset of aGVHD. The median time to onset of aGVHD was 47 days (range: 15–99 days). The median post-transplant chimerism recorded for comparison with the no aGVHD patients was 57 days. The aGVHD cohort had significantly lower CD3 recipient ( $P=0.005$ ) and higher CD3 UCB engraftment ( $P=0.006$ ). All other fractions, including the CD33 chimerisms, were not significantly different between the two cohorts. The aGVHD vs no aGVHD cohorts were further compared by degree of HLA mismatch (4–6 out of 8 vs 7–8 out of 8). The frequency of aGVHD was similar in the 4–6 out of 8 (23/62, 39%) and the 7–8 out of 8 (8/28, 28%) groups. Within these subgroups, CD3 UCB chimerism was higher for those with aGVHD ( $P=0.03$  and  $P=0.03$ , respectively). Conclusion The onset of aGVHD in haplo-cord transplantation is associated with a significantly higher CD3 UCB chimerism and lower CD3 recipient chimerism. Higher UCB chimerism may indicate that full UCB chimerism poses a higher risk of aGVHD development. Vice-versa persistent recipient chimerism may protect from acute GVHD.

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**Disclosure of conflict of interest:** None.

#### P178

##### Effect of single nucleotide polymorphism (SNP) in the interleukin-6 receptor gene on serum levels of CRP, IL-6 and soluble IL-6R and on the outcome after allogeneic stem cell transplantation

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IL-6 is a pleiotropic cytokine with both pro- and anti-inflammatory properties (Scheller 2014). The proinflammatory properties are mediated through trans-signaling that depends on the soluble IL-6 receptor. IL-6 trans-signaling is involved in several autoimmune diseases and in regulation of tissue regeneration of the GI-tract. Specific SNPs in the IL-6 receptor

have been associated with increased baseline CRP levels, severity of autoimmune diseases and response to interleukin-6 inhibition in rheumatoid arthritis. So far little is known about the role of trans-signaling in graft-versus-host-disease (GVHD). In this study we investigated how 4 specific SNPs in the IL-6 receptor influence pretransplant levels of CRP, IL-6 sIL-6R and the risk of grade II–IV acute GVHD in allogeneic stem cell transplantation (ASCT) in patients with family donor. DNA was available for 103 patients (65 male, 40 female median age 48, range: 15–70) and 101 donors, that underwent ASCT with a matched related donor (97 sibling) at Haukeland University Hospital in the period 2006–2016. The majority received conditioning with either ByCy (79) or FluBu (17) and only 2 patients were transplanted with TBI-based conditioning. Four different SNPs in the IL-6R gene (rs2228145, rs4845617, rs4845618, rs4845374) were chosen on the basis of (i) documented or suspected roles in autoimmune disorders; and (ii) allele frequency between 0.10–0.49 and  $R^2 < 0.5$  between the different SNPs. Genotyping was done using KASPar assays with ViiA7 instrument (Life Technologies). The overall genotype call rate was 98%. No departures ( $P$ -values  $< 0.01$ ) from Hardy-Weinberg equilibrium were observed. Pretransplant serum levels of IL-6, sIL-6R and were analyzed with Bio-Plex kits (Bio-Rad, Hercules, USA). Both serum and DNA analyses were performed in duplicates. Patients being homozygous for the rs4845618 minor allele had significantly higher pretransplant serum sIL6R levels but lower CRP levels compared with patients homozygous for the major allele. The overall incidence of aGVHD requiring high-dose steroid treatment (Grade II gastrointestinal, grade III–IV liver and skin) in the cohort was 48%. When analyzing the conventional clinical and laboratory parameters only transplantation with a non-sibling donor was associated with increased risk of aGVHD ( $P$ -value  $< 0.01$  HR 3.20 confidence interval 1.42–7.17). The presence of the rs4845617 in donor or recipient was associated with a significant increase in the rate of aGVHD ( $P$ -value 0.027 HR 1.93 confidence interval 1.08–3.47). The SNP rs4845617 ( $P$ -value 0.04 HR 1.86, confidence interval 1.04–3.35) was also significant in an adjusted model including both donor type and rs4845617. None of the evaluated SNPs were associated with an increase in early or late TRM and did not influence OS either. This study suggests that SNPs in the IL-6R influence pretransplant biochemical characteristics and clinical outcomes after ASCT. Future studies investigating the effect of IL-6 inhibition as GVHD prophylaxis or treatment should include analyses of IL-6 receptor SNPs to investigate their possible influence on treatment outcomes.

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**Disclosure of conflict of interest:** None.

#### P179

##### Previously published

#### P180

##### Efficacy of mycophenolate mofetil at low dose vs high dose in a triple immunosuppressive regimen in allogeneic hematopoietic stem cell transplant

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Graft-versus-host disease (GVHD) continues to be the major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplant (Allo-HSCT). The prophylaxis scheme varies according to the center and the country. In our institution we use triple-prophylaxis based on cyclosporin A

TABLE 1. Comparison between doses of MMF.

	MMF 500 mg BID until the day +35 post transplant	MMF 15 mg/kg BID until the day +180 post transplant	<i>p</i>
	n (%)	n (%)	
Acute (GVHD)	11 (19.6)	8 (23.59)	0.661
Chronic (GVHD)	11(19.6)	8(23.5)	0.661
Febrile neutropenia	36(64.3)	29(85.3)	0.031
<b>Infections in the first 100 days.</b>			
Viral	2(3.6)	5(14.7)	0.060
Bacterial	21(37.5)	16(47.1)	0.372
Fungal	2(3.6)	1(2.9)	0.871
<b>Neutrophil Recovery</b>			
Mean (S.D.)	13.56(6.43)	14.68(4.77)	0.003
Range	9-44	10-30	

(CyA), metrotrexate (MTX), and mycophenolate mofetil (MMF). This scheme has been used for more than one decade in Asian centers where it has proven adequate effective and safe to prevent GVHD. We evaluated 90 patients undergoing allogeneic hematopoietic stem cell transplantation treated at the National Cancer Institute from January 2010 to December 2015. The triple-prophylaxis scheme consists in CyA (adjusted serum levels, MTX (5 mg/m<sup>2</sup> days +1, +6, +11, +18) and we evaluated different doses of MMF, one of them includes 500 mg BID × 35 days and the other has high doses (15 mg/kg BID × 180 days), as GVHD prophylaxis. The response characteristic was analyzed using the Pearson test, Fisher's exact test on categorical variables and Student's *t*-test, Mann-Whitney *U* on continuous tests. Kaplan-Meier method was used to estimate the probabilities of OS, SLE with the differences compared by the log-rank test. We analyzed 90 patients with median of age of 30 years (range: 15–64), 60% male gender, all were transplant with peripheral blood progenitor cells as a source. 52.2% were acute lymphoid leukemia and 25.5% acute myeloid leukemia, 12.2% chronic myeloid leukemia, 3.3% myelodysplastic syndromes, 3.3% aplastic anemias, 2.2% non-Hodgkin's lymphomas and 1.1% Hodgkin's lymphomas. Myeloablative conditioning was used in 82% (BUCY, CFM-GAT) and 18% Reduce intense Conditioning (FLUBU, FLUCY, FLUCY-GAT), 94.4% related HLA compatibility. MMF 500 mg twice daily (BID) for 35 days (Group 1) and of MMF 15 mg/kg BID for 180 days (Group 2). In the group 2 the 85.3% developed febrile neutropenia vs 64.3% in group 1 (*P*=0.03). The frequency of GVHD was 19.6% group 1 vs 23.5% group 2 (*P*=0.6), chronic GVHD was 23.5% vs 19.6% respectively (*P*=0.7). At the moment of analysis 58.9% vs 26.5% were free of disease (*P*=0.01). There no difference at 5-year Overall survival was 37% (group 1) vs 49% (group 2) (*P*=0.85), neither free-survival disease (*P*=0.85). The MMF regimen shows non-inferiority scheme for GVHD. The low doses and for shorter administration did not show differences in the incidence and severity of acute or chronic GVHD, OS, DFS compared to the MMF regimen at 180 days with high doses. The high doses shows higher incidence of febrile neutropenia, but there were no differences in documented infections.

**Disclosure of conflict of interest:** None.

## P181

### Evaluating peritransplant albumin decline as a predictor of acute graft versus host disease

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A protein-losing enteropathy can develop due to conditioning regimen related gut toxicity and can cause albumin decline during peritransplant period in allogeneic stem cell transplantation (AlloHCT). Damaged intestinal mucosal barrier results in alloactivation of donor T cells and this situation is considered a primary event in the pathogenesis of acute graft-versus-host disease (aGVHD). Peritransplant albumin decline, as a result of conditioning regimen related protein-losing enteropathy, may predict aGVHD(1). In this retrospective study we tested this hypothesis. We evaluated 249 patients who received AlloHCT between 2011 and 2016. Albumin decline from the day of conditioning initiation until its nadir in the first 2 weeks of post-transplant period was calculated as delta albumin. Acute GVHD was proven by biopsy in all patients. Chi square and Mann-Whitney test were used for statistical analysis. Patients' characteristics were shown in table-1. Acute GVHD was developed in 78 patients and severe aGVHD was developed in 15 patients. Delta albumin was not different between aGVHD patients and no aGVHD patients. Delta albumin was not related with severe aGVHD. Delta albumin was not different between patients who received myeloablative and reduced intensity conditioning regimens. When we used a cut-off value of 0.9 gr/dL for delta albumin, we could not find a relation between delta albumin and development of both aGVHD and severe aGVHD. We repeated the analysis for acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients who receive myeloablative conditioning regimen and we found the same results, there was no difference between aGVHD patients and no aGVHD patients in terms of delta albumin. There was a number of studies that used albumin as a predictive and prognostic marker in the setting of aGVHD. But albumin may decrease in patients due to many reasons like malnutrition, proteinuria, enteropathy, liver disease or being negative acute phase reactant. Because of albumin value can show variability between patients, albumin decline may be a more objective criterion. Rashidi *et al.* showed that 0.9 gr/dL decline in albumin may be a predictor of severe aGVHD in 88 patients who was diagnosed with AML and MDS and received myeloablative conditioning regimen. We repeat this analysis in our MDS and AML (*n*=98) patients but we couldn't find this relation. When we evaluated all our 249 patients, again there was no relation between delta albumin and development of both aGVHD and severe aGVHD. In conclusion, our study did not support Rashidi *et al.*'s findings. Because serum albumin level shows variability due to many reasons, it is hard to use albumin as a predictor of aGVHD.

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**Disclosure of conflict of interest:** None.

**Table-1: Patients' characteristics**

	n
Age (median)	440(17-71)
Gender (M/F)	150/99
Diagnosis (AML/ALL/Aplastic anemia/myelofibrosis/Lymphoma/CML/MDS/Multiple myeloma)	99/43/31/9/28/17/17/5
HLA mismatch (no mismatch/one mismatch/haploidentical)	160/70/19
Stem cell source (Peripheral blood/bone marrow/cord blood/peripheral blood plus bone marrow)	204/31/4/10
Conditioning regimen (myeloablative/reduced intensity)	182/67
aGVHD (skin/gut/liver)	46/38/1

**P182****Evaluation of various therapeutic strategies in management of sclerodermatous chronic graft-versus-host disease including extracorporeal photopheresis, imatinib and sirolimus- multicentre retrospective analysis of polish adult leukemia group (PALG)**

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Sclerodermatous chronic graft-versus-host disease (Scl-cGVHD) in its severe manifestation affects the patient quality of life and, due to complex pathomechanism, does not respond to standard immunosuppressive therapy—calcineurin inhibitors (CNI) with corticosteroid. Methotrexate (MTX) and rituximab appeared to be effective in some patients but the novel strategies, including extracorporeal photopheresis (ECP), imatinib, m-TOR inhibitors (for example, sirolimus) and ruxolitinib seem to become the real breakthrough. We retrospectively analysed data of 33 patients with Scl-cGVHD, who underwent allogeneic hematopoietic cell transplantation (HCT) between 2009–2015 in 5 transplant centres. The study group consisted of 32 patients with haematological malignancies and one with aplastic anaemia, 14 female and 19 male, with the median age 36 (18–64). Donors' median age was 40, with predominance of matched sibling donors (21 donors) and even distribution of the donors' gender. In 22 patients (67%) acute GvHD (aGVHD) was diagnosed with skin involvement observed in 19 ones. Acute GvHD directly progressed to cGVHD in 13 cases. In 11 patients (33%) cGVHD developed 'de novo' and in 2 cases cGVHD was induced by DLI. Median time from HCT to cGVHD diagnosis was 8 months and to Scl-cGVHD diagnosis- 32 months. Seven patients (21%) were scored as moderate cGVHD and 26 patients (79%) as severe cGVHD according to NIH-2014 cGVHD activity classification. In 14 patients sclerotic features had superficial form and in 19 ones deep sclerosis was observed. Chronic GvHD

manifestation in other organs includes: mouth (94%), joints and fascia (77%), liver (74%), eyes (64%), GI tract (33%) and lungs (21%). 26 patients were treated with ECP and/or sirolimus and /or imatinib with 80% response rate (complete-CR, partial-PR or minimal-MR). Median duration of ECP therapy, sirolimus and imatinib treatment was 20 months (2–40), 3 months (1–30), and 5 months (1–18), respectively. Sirolimus was added more likely (9 patients) as the first in case of suboptimal response to ECP after median 15 weeks and in 4 patients was subsequently replaced by imatinib with no favourable outcome in 3 cases. In 3 patients imatinib was initially used in combination with ECP therapy, leading to PR or MR. MTX without novel therapies was effective in 4 patients with limited skin involvement, 3 patients responded to MTX plus imatinib and 1 patient to MTX plus sirolimus. Two patients, after failure of other therapies, have been receiving ruxolitinib with improvement. Only 3 patient (15%) were non-responsive to ECP (progressive or stable disease), 4 patients (36%) to sirolimus and 4 patients (33%) to imatinib. Toxicity incidence was equally observed in case of sirolimus and imatinib and lead to the therapy discontinuation in altogether 4 patients. Infectious complications were observed in 20 patients (60%). ECP confirms to be the most effective therapeutic strategy in severe forms of Scl-cGVHD with favourable safety profile. Imatinib and sirolimus, targeting different fibrotic pathways, both play important role in non-responsive patients, improving the outcome in ECP and non-ECP group. In case of limited access to ECP, MTX remains to be beneficial in combination therapy of moderate Scl-cGVHD and an alternative to CNI.

**Disclosure of conflict of interest:** None.

**P183****Previously published****P184****Extracorporeal photopheresis (ECP) impacts on GvHD-relevant but not virus-specific and anti-leukemic immune cells**

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Post-transplant morbidity and mortality are majorly determined by GvL effect counter-balanced by GvHD. Treatment with systemic steroids represents the first-line therapy for GvHD, but is associated with increased incidence of infection and relapse. ECP can reduce the extent of GvHD while preserving anti-virus/-tumor activity. To elucidate this clinical phenomenon on an immunological level, we correlated clinical data with immunological findings in 20 patients under ECP treatment. Nine patients with acute GvHD (aGvHD) of the gut II–IV suffering from severe diarrhea were treated by ECP in addition to triple-drug immunosuppressive therapy. Furthermore, 11 patients with chronic GvHD (cGvHD) of the skin or lung despite triple-drug received ECP treatment. Patients were evaluated according to their individual response and clinical condition. Phenotypical analysis of different cellular subsets of patients and healthy donors was performed by multicolor flow cytometry. Functional properties of virus-specific CD8+ T and NK cells were evaluated by INF- $\gamma$ -ELISPOT and 51Cr-release assay. About 20 patients were treated by ECP in this study. However, two aGvHD and two cGvHD patients had to be withdrawn from ECP treatment after a few ECP cycles due to pancytopenia or poor clinical condition. For patients with aGvHD 8 up to 25 ECP cycles were needed for response. All patients achieving a complete response (CR) were still alive 1 year after initiating ECP therapy. Overall response, that is, CR or partial response (PR) according to NIH criteria, was obtained in 5 of 7 patients with aGvHD (71.4%) including CR in 3 of 7 (42.8%). Out of 9 cGvHD patients 7 (77.8%) reached PR, and 2 (22.2%) remained stable under ECP treatment. After 1 year, overall survival (OS) was 60% for aGvHD patients responding to ECP, while only 25% for non-responders. OS for cGvHD patients was 91%. During intensive ECP treatment for patients with aGvHD of the gut, the average stool volume and frequency decreased and consistency changed from loose to formed stool. Steroids could be tapered down to a mean of 22% of the initial dosage. cGvHD patients were stabilized under ECP treatment and steroid dosage could be reduced to a mean of 38%. Clinically responding patients showed increased numbers of regulatory cells including MDSCs, Foxp3+CD8+ and Foxp3+CD25+CD4+ Tregs, as well as CD4–CD8–CD3+ T, V $\delta$ 2+ T cells and regulatory B lymphocytes. Furthermore, loss of CD62L expression on effector cells like CD4+ TE, CD8+ TE, NK and NKT cells was observed under ECP treatment. Interestingly, ECP treatment did not dramatically influence the frequency of CD4+CD8+CD3+ T,  $\gamma\delta$  T cells and NKT cells, which possess anti-virus/-tumor function. ELISPOT and 51Cr-release assays revealed stable anti-viral activity of CD8+ T cells as well as functional cytotoxicity of NK cells. Moreover, CD8+ T, CD8+ TEM, CD62L+CD4+ TEMRA, CD56+CD3– NK and CD56brightCD16– NK cells could serve as reliable biomarkers for prediction of response to ECP.

**Conclusion:** ECP treatment might stabilize or even improve clinical situation of patients suffering from GvHD. In clinically responding patients an immunomodulation was observed in terms of increasing numbers of regulatory cells with loss of migratory capacity of effector cells while anti-virus/-leukemia T-cell function was preserved.

**Disclosure of conflict of interest:** None.

## P185

### Extracorporeal photopheresis affects dendritic cells by reducing total numbers and blunting cytokine production in patients with graft versus host disease

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Graft versus host disease (GvHD) and concomitant immunosuppression is a leading cause of morbidity and mortality post hematopoietic stem cell transplantation (HSCT). The pathophysiology of GvHD is complex, involving presentation of histo-incompatible antigen by activated recipient dendritic cells (DCs), activation and proliferation of donor T cells and resultant tissue damage. Extracorporeal photopheresis (ECP) is a second-line treatment for steroid refractory or dependent GvHD that facilitates the reduction of immunosuppression. ECP's mechanism of action is unclear and is likely to be multifaceted. Apoptosis of lymphocytes, induction of a Th2 favoured environment and increased numbers of regulatory lymphocytes have been implicated<sup>1</sup>. Although ECP has been shown to modify the function of *in vitro* monocyte-derived DCs<sup>2</sup>, its effect on primary (non monocyte-derived) DCs has not been studied. Our aim was to determine whether ECP had immediate or long-term effects on primary DC numbers or function. We enumerated monocyte and DC subsets (cDC1, cDC2 myeloid DCs and plasmacytoid DCs) in whole blood before, during and after ECP cycles, and developed a novel DC function assay, suitable for use on clinical samples. Four adults with immunosuppression withdrawal GvHD and four children with acute GvHD, received ECP during the study. All received ciclosporin GvHD prophylaxis and corticosteroid treatment at onset of GvHD. Children received  $\geq 1$  dose of Infliximab prior to starting ECP. Adults received two ECP treatments (one cycle) every 2 weeks and children received two ECP treatments (one cycle) weekly. Whole blood was taken before and after each cycle of ECP. TruCount flow cytometry analysis of whole blood was used to enumerate mononuclear leukocytes. To assess function, peripheral blood mononuclear cells were isolated by density centrifugation and stimulated with toll-like receptor agonists. Cell-specific cytokine production was then analyzed by flow cytometry. Samples were compared to healthy controls and pre-ECP samples. Median time to first ECP treatment from GvHD diagnosis was 33.5 days. No GvHD flares were experienced during study period. (1) Adult had a cycle of treatment delayed due to intercurrent pneumonia. Numbers of cDC2, pDCs and classical monocytes were significantly reduced after each ECP treatment in the adult group. DC numbers followed the same trend after ECP in the paediatric group but were not significantly different before and after ECP. This is perhaps due to initial lower DC count compared to adults in the children before the first ECP cycle. Functional analysis showed a reduction in cytokine production in DCs and monocytes in both groups over the course of ECP treatment. Our data support a cell-intrinsic effect of ECP on monocytes and DCs, with numerical and functional consequences. This may contribute to the beneficial effect of ECP both through reduction of inflammatory effector function and through modulation of interactions with other immune cells. Correlation with immunosuppression withdrawal and clinical events during treatment may provide further insight into the role of monocytes and DCs in GvHD and ECP, which may aid in the development of novel targeted therapies for GvHD.

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**Disclosure of conflict of interest:** None.

**P186****Extracorporeal photopheresis as early second-line treatment for patients with steroid-dependent or refractory acute graft-versus-host disease: a single-centre experience**

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Acute graft-versus-host disease (aGVHD) remains a severe complication of allogeneic haematopoietic cell transplantation (alloHCT). Corticosteroids as the backbone of initial therapy for aGVHD result in varied complete responses (25–69%). Traditional secondary treatments lead to profound immunosuppression without improved survival. On the basis of our experience in chronic GVHD, we aimed to prospectively assess the role of extracorporeal photopheresis (ECP) as early second-line treatment in steroid-dependent and refractory aGVHD. We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II–IV aGVHD post alloHCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5 mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine–methotrexate in myeloablative and cyclosporine–mycophenolate mofetil in reduced toxicity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: two sessions per week for 1 month, one session per 2 weeks for 3 months, evaluation of response and one session per month for 6 months. We studied 20 patients, aged 35 (18–65), post alloHCT with myeloablative (14), reduced toxicity (4) and intensity (4) conditioning, from sibling (3), matched (8) or one locus mismatched (8) volunteer unrelated and haploidentical (1) donors. Disease risk index was high (10), intermediate (9) and low (1). Acute GVHD was observed at day +17 (8–50) in 15 patients, late onset at +130 (110–160) in 4 patients and induced at +38 post donor lymphocyte infusion in a relapsed AML patient. Skin, intestine and liver involvement was evident in 6 patients, skin and intestine in 10 and skin only in 4 patients. Nine patients (2 with GrII, 7 with GrIII aGVHD) were steroid-dependent and 11 (8 with GrIII, 3 with GrIV) steroid-refractory. ATG was administered simultaneously with ECP initiation in six refractory patients that further developed EBV reactivation ( $P=0.032$ ) treated pre-emptively with rituximab. ECP was commenced at day +51 for 15 (4–20) sessions. The majority of patients (16/20) presented partial (6), very good (9) or complete (1) response to ECP. With 8.3 (1.7–51) months of follow-up, immunosuppression was reduced in 10/20 and ceased in 1 patient. Clinically significant bacterial infections were found in 17 patients, fungal in 2, CMV and EBV reactivation in 14 and 13, respectively, and other viral in 5 patients. Cumulative incidence (CI) of chronic GVHD was 77.4 at 1 year. One-year CI of aGVHD-related mortality was 21%. One-year overall survival (OS) was 53% and significantly increased in steroid-dependent vs refractory patients (80% vs 36%,  $P=0.039$ ). Reduction of immunosuppression ( $P=0.008$ ) and response to ECP ( $P=0.034$ ) were associated with improved OS, irrespectively of other factors. Our data indicate that ECP should be considered early in the course of steroid-dependent or refractory aGVHD, before irreversible end organ damage has been established. Optimal timing of intervention, frequency, duration and tapering schedule of ECP remain important unanswered questions.

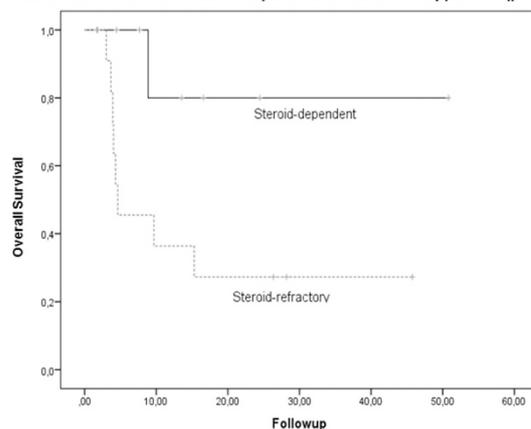
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[P186]

Increased overall survival in steroid-dependent versus refractory patients ( $p=0.039$ )



**Disclosure of conflict of interest:** None.

**P187****Extracorporeal photopheresis for treatment of chronic graft versus host disease**

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Allogeneic stem cell transplantation represents a curative approach to many hematologic disorders. Graft versus host disease (GVHD) is a complication with significant morbidity, mortality and decreased quality of life. Extracorporeal photopheresis (ECP) represents possible treatment approach. Mononuclear cells (MNC) collected by apheresis are photosensitized with 8-methoxypsoralen *ex vivo*, irradiated with UVA and transfused back to the patient. **Aim of the study:** Evaluation of patients treated with ECP for GVHD at our center. Thirteen patients (8 females and 5 males, median age 44 years) were treated with ECP. About 12 patients (pts) had matched sibling donor and 1 patient had unrelated donor. About 10 pts had sclerodermic form of GVHD, 2 had concomitant pulmonary GVHD, 2 had pulmonary GVHD alone. One pts had mild, seven moderate and five severe GVHD according to NIH. About 11 patients were treated with steroids. MNC separation was prepared on COBE Spectra and Spectra Optia (Terumo BCT, USA). 8-methoxypsoralen was added, irradiation was done on Macogenic G2 (Macopharma, Mouvoux, France). About 696 procedures in 13 pts were performed (median 41 procedures, 12–120 procedures). The schema was as follows: ECP on 2 consecutive days every 2–3 weeks first 3 months with subsequent increase in interval. Median follow up was 35 months. In sclerosing form two pts reached CR, six pts PR, one is stable and one patients progressed. In pulmonary GVHD one reached CR, two partial improvement, one is stable. Seven pts are still alive, six died (two due to relapse, one secondary malignancy and three infections). It was possible to withdraw steroids in 10 pts. Adverse events were clinically negligible. ECP is an effective treatment for chronic GVHD. Especially sclerodermic form responds to ECP very well. It is safe and well tolerated procedure with minimal toxicity. Supported by PRVOUK P-37.

**Disclosure of conflict of interest:** None.

[P188]

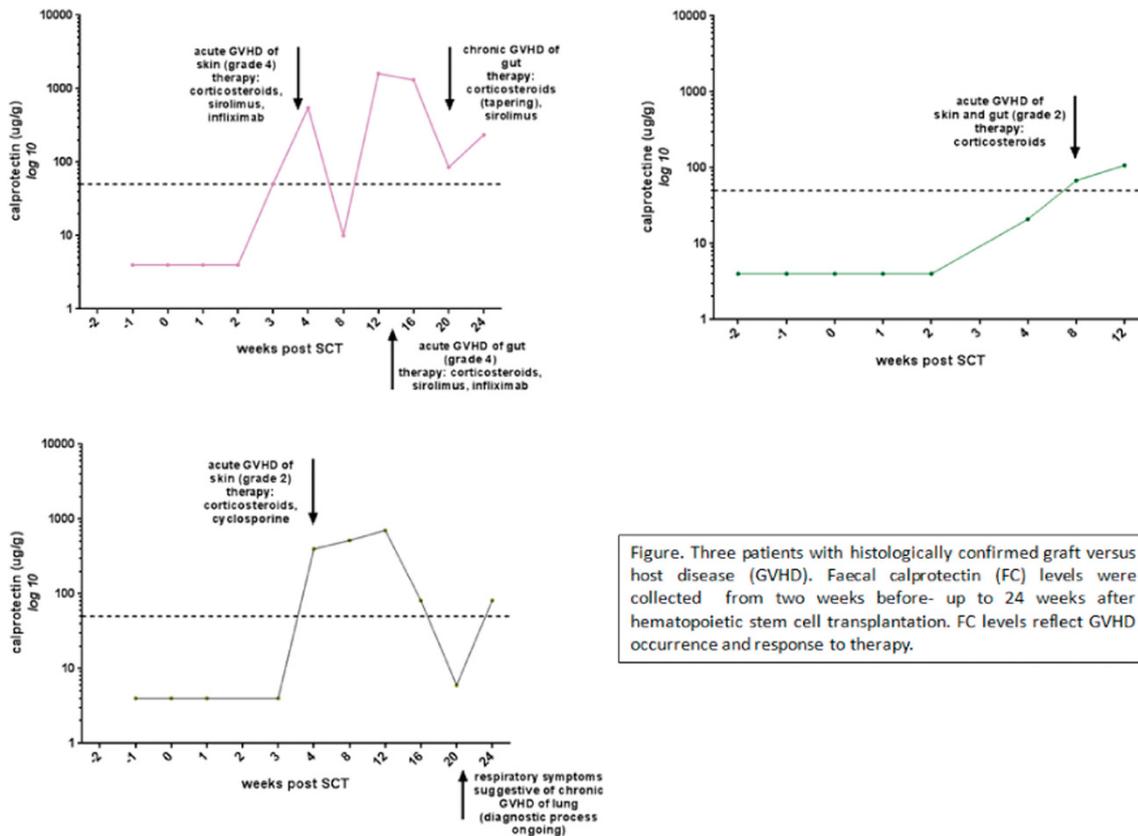


Figure. Three patients with histologically confirmed graft versus host disease (GVHD). Faecal calprotectin (FC) levels were collected from two weeks before- up to 24 weeks after hematopoietic stem cell transplantation. FC levels reflect GVHD occurrence and response to therapy.

**P188**  
**Faecal calprotectin as non-invasive marker for graft versus host disease after paediatric allogeneic haematopoietic stem cell transplantation: an interim analysis**

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Allogeneic haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment option for children with a variety of haematological, oncological and immunological diseases. Graft versus host disease (GVHD) represents a major cause of post-transplantation mortality and morbidity affecting multiple organs including skin, gut, liver and lungs. GVHD is considered a succession of inflammation and donor T-cell activation initiated by translocation of gastro-intestinal micro-organisms through impaired mucosal barriers after chemotherapeutic conditioning and/or infection. Diagnosis of GVHD is based on clinical symptoms and histological findings, necessitating invasive and potentially harmful procedures including endoscopy and biopsy. As yet, no non-invasive markers are available for diagnosis or treatment monitoring in children with GVHD. Faecal calprotectin (FC) reflects intestinal mucosal inflammation of any origin. In the setting of allogeneic HSCT in adults, FC has shown to be a marker for acute (steroid-resistant) GVHD. We aimed to evaluate the feasibility of prospective FC measurement as a non-invasive marker for diagnosis and treatment in children with GVHD. A prospective, observational, single centre study was started in July 2015. By December 2016, 21 paediatric allogeneic HSCT patients (age 0–17 years) were included after informed consent. Faecal samples were collected from 2 weeks before to 6 months after HSCT. FC levels were measured by Eia, according to manufacturer's instructions. Clinical symptoms were prospectively evaluated and managed according to local

guidelines. If GVHD was suspected on clinical grounds, histological confirmation was obtained. First-line therapy for GVHD consisted of corticosteroids. In case of steroid-resistant disease, more advanced immune modulation was applied. A total of five patients developed histologically confirmed GVHD: acute GVHD of skin and gut ( $n=2$ , one patient with steroid-resistant disease), acute GVHD of skin only ( $n=1$ ); chronic GVHD of lung only ( $n=1$ ) and acute GVHD of skin followed by chronic oromucosal GVHD ( $n=1$ ). Without exception and regardless of gut involvement, GVHD occurrence was accompanied by rises in FC levels to values  $> 100 \mu\text{g/g}$  (range: 108–1600  $\mu\text{g/g}$ ). FC levels correlated with clinical and histological grading. Moreover, adequate response to therapy was consistently reflected by return of FC levels to values  $< 100 \mu\text{g/g}$ . Sensitivity of FC levels to diagnose GVHD was poor due to increased FC levels in patients with post-transplant complications other than GVHD such as viral reactivation and pulmonary or gastro-intestinal infections. FC levels reflect GVHD occurrence and correlate with clinical and histological grading in paediatric allogeneic HSCT patients. FC levels increase in case of GVHD regardless of gut involvement, supporting a central role for (subclinical) intestinal inflammation in GVHD initiation. Although, in this interim analysis, FC lacks sensitivity to diagnose GVHD, FC may serve as a non-invasive marker for monitoring therapy response and, thereby, reduce the need for repeated invasive procedures including endoscopy and biopsy.

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**Disclosure of conflict of interest:** None.

**P189**

**First-line extracorporeal photochemotherapy (ECP) for acute GvHD after allogeneic stem cell transplantation**

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Acute graft-versus-host disease (aGVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT), and glucocorticoids are typically used as first-line treatment. The aim of our study was to evaluate the effect of first-line ECP +/- steroid therapy in order to reduce the incidence of infections and toxicity. From December 2010 to January 2016, 48 of 180 pts (27%), were diagnosed with aGVHD grade ≥2 following alloSCT. 40 pts were treated with ECP +/- steroid as first-line therapy. About 8 (20%) pts were treated with ECP only and 32 (80%) with ECP + steroid 1–2 mg/kg/day. We compare this cohort with an historical group of patients, transplanted between 2001 and 2011, who were treated with steroid only for grade 2–4 aGVHD (n = 23 out of 130). The two cohorts were well balanced in terms of median age (P=0.4), disease type (P=0.9), disease status (P=0.09), graft source (P=0.2), conditioning regimen (P=0.1) and HCT-CI (P=0.3). There were more female patients (P=0.03) and more haploidentical transplant (Haplo-SCT) (P=0.0005) in the cohort treated with ECP +/- steroid. ECP was performed using the offline technique, and was started as soon as possible with a treatment schedule consisting of four rounds of two procedures per week, three rounds of two procedures every other week and finally two procedures every month. Steroid

was tapered as soon as possible after starting ECP. The clinical response was evaluated at day +28. Median follow-up for alive patients was 28 months for ECP group and 97 months for control group. There was no difference in terms of median time of aGVHD onset (38 vs 39 days) and number of pts with grade 2 or 3–4 aGVHD (Figure 1). ECP was started after a median of 4 (0–30) days from aGVHD diagnosis. Every patient underwent a median of 19 (2–83) ECP procedures, during a median time of 6 months. On day 28 after starting aGVHD treatment with ECP +/- steroid, 24 pts (70%) achieved CR or PR, 10 pts did not respond and 2 experienced aGVHD relapse to front-line therapy. One year cumulative incidence (CI) of aGVHD relapsed/refractory was 28.5% for ECP +/- steroid. These percentages were not different from the cohort receiving steroid alone. CI of moderate–severe cGVHD was lower in the ECP group, probably due to the higher frequency of Haplo-SCT with PT-Cy in the ECP group. About 100 days after aGVHD onset, CI of infection (49% vs 74%), especially CMV reactivation (34% vs 67%), was lower in the ECP group, but was not statistical significant. ECP allowed a faster taper of steroid: 17 (3–98) vs 75 days (23–338) (P < 0.0001). Overall survival, progression-free survival, non-relapse mortality and CI of relapse rates did not differ in the two groups. In multivariate analysis, visceral involvement by aGVHD was associated with an increased risk of failure to front-line therapy (HR: 5.5; range: 0.7–41; P=0.09).

This observational study suggests that the overall response rate of ECP +/- steroids is similar to steroid alone for front-line treatment of grade 2–4 aGVHD, but is potentially associated with lower incidence of infection, and in particular of CMV reactivation. A prospective phase 2 clinical trial is warranted to address whether augmentation with ECP may be beneficial for aGVHD front-line treatment.

[P189]

Acute GVHD characteristics and outcome

Characteristics	Cohort Steroid	Cohort ECP +/- steroid	P value
	23	40	
aGVHD grade			
Grade 2	17 (74%)	33 (82%)	0.5
Grade 3-4	6 (26%)	7 (18%)	
aGVHD median day	38 (8-102)	39 (13-177)	0.1
Response			
CR	19 (83%)	24 (60%)	0.1
PR	1 (4%)	4 (10%)	
SD/PD	3 (13%)	12 (30%)	
# pts aGVHD relapsed/refractory Y/N	5/18	14/26	0.3
1y-CI aGVHD relapsed/refractory	22.7%	28.5%	0.58
# pts cGVHD moderate-severe Y/N	11/10	8/25	<b>0.08</b>
5y-CI cGVHD	60%	22%	<b>0.09</b>
Steroid stop (median day)	75 (23-338)	17(3-98)	<b>&lt;0.0001</b>
# pts with Infections Y/N	17/23 (74%)	19/40 (47%)	<b>0.06</b>
# Infections			0.1
Bacterial	7	9	
Fungal	0	3	
Viral	31	28	
Recurrent CMV infection	5	5	
100d-CI Infections	74%	49%	0.1
100d-CI CMV infection	67%	34%	0.1
1y-NRM	26%	28%	0.9
5y-PFS	40%	44%	0.2
5y-CI disease relapse	25%	24%	0.9
5y-OS	42%	45%	0.5

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- Disclosure of conflict of interest:** None.

## P190

### Graft-versus-host disease in a child with neuroblastoma after autologous bone marrow transplantation

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A 4-year-old girl with neuroblastoma received autologous stem cell transplant (ASCT), followed by antibiotic prophylaxis and filgrastim. Her transplant preparative regimen consisted of busulfan and melphalan. Engraftment of neutrophil took place on day 11 after ASCT. Twentieth day after ASCT, she experienced nausea and diarrhea. There was neither skin rash nor elevation in liver enzymes. The diarrhea continued to worsen day by day and reached to a daily volume of 1500 ml/m<sup>2</sup>. Infectious studies for stool and blood including testing for influenza A and B, Parainfluenza, Adenovirus, Epstein–Barr virus, Amebiasis, Cryptosporidium Parvum, Cytomegalovirus, Clostridium difficile, Salmonella, Campylobacter, Yersinia and Shigella were all negative. Colonoscopy and endoscopy were performed by an experienced pediatric gastroenterologist and findings were suspicious for severe graft versus host disease (GVHD). Colonoscopy and rectoscopy revealed severe inflammatory changes, friability and patchy dark exudates on the mucosa of rectum. Endoscopy revealed erosions, ulcers in the esophagus and a pale mucosal surface with reticulated submucosal vessels accompanied with erosion and erythema in the antrum of stomach. Grade 3 GVHD was confirmed by pathologic analysis that revealed diffuse crypt dropout and mucosal erosion on rectal mucosal biopsy. Mucosal erosions, apoptosis of epithelial cells and small lymphocytic infiltration of the lamina propria were found on duodenal biopsy. After these results, we started methylprednisolone intravenously at a dosage of 2 mg/kg/day. On the fourth day of treatment we increased the dosage to 5 mg/kg/day and added cyclosporine to treatment. Because of unresponsiveness to treatment we decided to administer third-party mesenchymal stem cells (MSC) ( $1 \times 10^6$  CD73+/CD105+ cells per kg). These were given intravenously at day +49 ASCT as single infusion. The second dose was given at day +56. Within 5 days after first application of MSCs, the frequency of diarrhea decreased to one-third. At day +16 after second dose of MSCs, the patient's stool became nearly normal. We tapered the steroids first and stopped cyclosporine at +92th days after ASCT. **Discussion and conclusion:** To our knowledge, this is the second case report of spontaneous severe autologous GVHD in a child with a solid tumor malignancy. Regarding the pathogenesis of autologous graft-versus-host disease, there may have multiple causes for the loss of tolerance to self because of disrupted immune system. Alteration of T regulatory cells by previous chemotherapy may be key point. Endogenous cells that survive conditioning and assist in post-transplant maintenance of self-tolerance may be affected. Microchimerism due to maternal cells transmitted during fetal development and persisting throughout adult life has also been postulated as a cause. However it is not very clear for factors that may contribute to the pathogenesis of this rare disease. Autologous GVHD has the potential to cause critical illness in the hematopoietic stem cell transplantation patient population. In patients with multiple myeloma some experts report pathologically verified gastrointestinal GVHD as high as 6%. Responses to steroids are variable. However, a significant proportion improve dramatically after early therapeutic intervention. So clinicians and pathologists should be aware in suspecting and recognizing GVHD in patients with diarrhea to guide therapy as soon as possible.

**Disclosure of conflict of interest:** None.

## P191

### Graft-versus-host disease in paediatric hematologic malignancies receiving best unrelated donor BMT compared to ex vivo T-cell depleted haploidentical stem cell transplant

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Pediatric patients with hematologic malignancies receiving transplant from the best unrelated donor (BUD, matched unrelated donor and mismatched unrelated donor) remain at risk of severe acute and chronic graft-versus-host disease (aGVHD and cGVHD). The early reports of lower GVHD risks in related T-cell depleted (TCD) haploidentical stem cell transplants (HaploSCT) is a safe and acceptable alternative. **Aim:** We retrospectively compared the risk of GVHD in patients who underwent BUD Blood/Marrow Transplant (BMT) with patients who received HaploSCT for hematologic malignancies. We searched electronic and paper records of all children who underwent transplant from 05 September 2005 to 16 August 2016 for hematologic malignancies. The parents/patients have signed consent in the use of their data. There were a total of 67 evaluable BMTs in this 10-year period. There were a total of 39 BUD BMT from 2005 to 2013 and 28 HaploSCT from 2012 to 2016. BUD BMTs receive GVHD prophylaxis (Cyclosporine or Tacrolimus and Methotrexate). HaploSCT patients did not receive additional GVHD prophylaxis aside from the *ex vivo* T-cell depletion (TCD) with Clinimacs system. Of the BUD BMTs 36 out of 39 patients engrafted, 92% of which had GVHD. The 8% who did not have any GVHD, relapsed. Eighty one percent had aGVHD, of which majority (51%) were grade 2 (Table 1). Three patients did not have aGVHD (10/10 MUD and two cord blood grafts), but developed cGVHD. One of the patients who had grade 4 aGVHD died and one still has intermittent cGVHD 12 years post BMT. There were 55% who had cGVHD. Currently, all of our HaploSCT receive a CD3/CD45RA TCD grafts (N=14). The depletion techniques for the 14 others were either CD3TCD, CD3/ CD45RA /TCRab TCD+CD34+/CD45RA TCD; TCRab TCD. All 28 patients who received HaploSCT engrafted and 57% had aGVHD (56% grade 1) (Table 1). We noted that some of the patients presented with non-classical aGVHD signs (upper gut GVHD, oral GVHD, blood). There were no patients who presented with grade 4 aGVHD. cGVHD in the cohort was 35%. Of note, 35% of patients did not have any GVHD and did not receive any form of immunosuppression post BMT. Only eight patients received further immunosuppression for aGVHD, median duration 131 (range: 91–344) days. At the time of this report, there are four patients with aGVHD still receiving immunosuppression all < 100 days and all on tapering doses. HaploSCT using *ex vivo* TCD techniques has a lower risk of GVHD with comparable, if not superior outcome to BUD. The degree and duration of immunosuppression is also much less. This may translate to earlier immune reconstitution and less viral reactivation.

[P191]

Table 1: Percentage of acute and chronic GVHD

Type of BMT	Grade/ Extent	% Acute GVHD				% Chronic GVHD		
		Grade1	Grade 2	Grade 3	Grade 4	Limited	Extensive	
BUD	N=36	21%	51%	14%	14%	N=20	60%	40%
HaploHSCT	N=20	57%	25%	18%	0%	N=10	60%	40%

**Disclosure of conflict of interest:** None.

**P192****GVHD and circulating endothelial cells: it takes two to tango**

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) offers a potential cure for several hematological diseases, but it is burdened by severe life-threatening complications, being GVHD the major cause of morbidity and mortality. Recently, more have been understood of the physio-pathologic relationships between endothelium and graft-versus-host disease (GVHD), showing that vascular endothelium is an early phase target of GVHD. In recent years, the direct count of circulating endothelial cells (CEC) has emerged as a valuable biomarker of endothelial damage in a variety of disorders. However, due to their rareness and complex phenotype, different published techniques have showed variable degrees of uncertainty, reporting a wide range of CEC values in healthy subjects. By means of the commercially available rare cell isolation platform CellSearch system, for CEC identification and count, we correlated CEC count changes to GVHD onset and response to treatment in allo-HSCT patients. CEC were analysed in 90 allo-HSCT patients (37 AML, 15 ALL, 11 HD, 4 NHL, 4 CLL, 5 MDS, 4 CMS, 8 MM, 2 SAA) at the following time points: T1 (pre-conditioning), T2 (pre-transplant), T3 (engraftment), T4 (day+28 or onset of GVHD), T5 (1 week after steroid treatment). The median CEC/mL at T1 was 24 (range: 2–786), in comparison to a value of 2 (range: 1–14) in healthy controls (p 0%: OR 4.2, 95% CI 1.6–10.8; P=0.002). We confirm that CEC count represent a valid biomarker to monitor endothelial damage in patients undergoing allo-HSCT and can be a valuable tool in supporting the diagnostic definition of GVHD and in monitoring responsiveness to treatment. Moreover, the use of the CellSearch system can be crucial in order to move routinely CEC monitoring into clinical practice of allo-HSCT.

**Reference**

ClinicalTrials.gov NCT02064972.

**Disclosure of conflict of interest:** This research was conducted with the support of the Investigator-Initiated Study Program of Janssen Diagnostics, LLC to CA. KB is employee of Janssen Diagnostics.

**P193****High-dose cyclophosphamide for GVHD prophylaxis in mismatched unrelated donor transplantation**

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Results of HLA mismatched unrelated donor (MMUD) hematopoietic cell transplants (HCT) are worse than results of fully matched HCT due to higher risk of GVHD, infection and graft failure. ATG during conditioning reduces incidence of GVHD but can increase risk of infection and relapse. High doses posttransplant Cyclophosphamide (2 × 50 mg/kg) prevent GVHD in haploidentical HCT. We initiated this approach instead of ATG in HCT from one allele or antigen mismatched unrelated (7/8)MMUD-HCT in 2014. Here we present outcome of 21 patients (Cy-group) transplanted between 2014 and 2016, comparing it with outcome of 54 patients transplanted

between 2010 and 2016 from 7/8 MMUD with ATG-F (Fresenius) 40 mg/kg given during conditioning. 21 patients in Cy-group (12 males, 9 females) were transplanted from MMUD mismatched for HLA (A-8, B-3, C-5, DR-5). About 14 patients had AML, 2 MDS, 2 CLL, 1 CML, 1 ALL and 1 MPS. Med. age of patients was 43 years (25–60). About 16 patients received myeloablative (Flu-175 mg/m + iv Bu 12.8 mg/kg) and 5 nonmyeloablative (Flu-175 mg/m + Mel 100–140 mg/kg + TT 5 mg/kg) conditioning. About 18 patients received PBPC and 3 BM as a graft. Graft versus host prophylaxis consisted of Cyclophosphamide (50 mg/kg aBW) on D+3 and +5, Cyclosporine A from D 0 and MMF from D+1. All patients received antibacterial, antifungal, HSV and PCP prophylaxis. Historical control (ATG) group consisted of 54 patients (32 males, 22 females), med. age 54 y (19–65) who had MMUD-HCT for AML-21, MDS-9, NHL-5, MF-5, ALL-4, CLL-4, CML-2, H.D-1, SAA-1, MPS-1 and MM-1. There were 13 mismatches for A, 11 for B, 20 for C and 10 for DR. Myeloblastic conditioning was used in 32 and nonmyeloablative in 22 patients. All patients received ATG-F 20 mg/kg × 2 given D-2 and -1. Cyclosporin was initiated D-2 and MMF D-1. All patients received anti-infectious prophylaxis as described previously. Three of 21 patients from Cy group died so far. Two of them due to relapse and one due to toxicity and infection during aplasia. Five patients relapsed. Two achieved CR after DLI and one is alive in relapse expecting second HCT. About 18 patients are alive, 17 of them in CR. Eight patients experienced aGVHD (Gr.I-3, Gr.II-5, Gr.III-0, Gr.IV-0) and eight developed clinically mild cGVHD). About 22 patients from ATG group are alive 11–80 m (med. 32 m) postHCT. About 32 patients died 1–74 m posttransplant (med. 16 m) due to VOD, GVHD, infections and relapse. 100-day mortality is 5% (1/21) in Cy group and 19% (10/54) in ATG group. One year mortality is 14% (2/14) in Cy and 30% (13/44) in ATG group. Patients from Cy group have 78% probability of OS at 24 months postHCT vs 47% from ATG group. Cyclophosphamide 2 × 50 mg/kg instead of ATG Fresenius (40 mg/kg) for GVHD prophylaxis reduced 100-day and 1-year mortality, and improved probability of 24 m OS significantly in our cohort of patients. This approach seems to be safe and effective in 7/8 MMUD-HCT.

**Disclosure of conflict of interest:** None.

**P194****High transplanted CD34+ cells are not associated with beneficial effect on graft-versus-host disease-free, relapse-free survival (GRFS) after allogeneic hematopoietic cell transplantation**

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The success of allogeneic hematopoietic cell transplantation (allo-HCT) is comprehensively assessed by individual comorbidity, relapse, graft-versus-host disease (GVHD) and death. Besides, inconsistent results have been reported regarding the dose of CD34+ cells. In the current study we have addressed the issue of the potential effect of stem cell dose on the GVHD-free/relapse-free (GRFS) associated with CD34+ cells doses. We retrospectively reviewed the medical records of the 255 patients who received allo-HCT for acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS) and acute lymphoblastic leukemia (ALL) between 1998 and 2013 in Kyungpook National University Hospital. The GRFS included grade 3–4 acute GVHD, systemic therapy-requiring chronic GVHD, relapse or death. The patients were reclassified into two groups according to the targeted CD34+ cell doses (6 × 10<sup>6</sup> per kg) by KNUH protocol. A lower CD34+ group (n = 165, 64.7%), patients who underwent allo-HCT with CD34+ cell dose < 6 × 10<sup>6</sup> per kg; and a higher CD34+ group (n = 90, 35.3%) patients who underwent allo-HCT with CD34+ cell dose ≥ 6 × 10<sup>6</sup> per kg. The median age at transplant was 38.5 years (range: 15–68 years) and male was 111 patients (44.4%). Primary diseases for allo-HCT were AML/MDS (n = 175, 70%) and ALL (n = 75, 30%).

One hundred forty-three patients (57.2%) were in CR1 (complete remission), 25 (10%) in further CR and 87 (33.2%) in relapsed and refractory status. One hundred seventy-one patients (68.4%) received myeloablative conditioning regimen. GVHD prophylaxis consisted of methotrexate and cyclosporine A or MTX and Tacrolimus. The median dose of CD34+ cell was  $3.94 \times 10^6$  per kg (range:  $0.46-6 \times 10^6$  per kg) in lower CD34+ group and  $7.54 \times 10^6$  per kg (range:  $6.01-20.6 \times 10^6$  per kg) in higher CD34+ group. There was no significant difference in neutrophil, platelet engraftment between two groups. The incidence of chronic GVHD was more frequent in higher CD34+ group (32.9% vs 48.2%,  $P=0.042$ ). The median follow-up duration was 18.1 months, with a range of 0.2–209.7 months. The 1-year overall survival (OS), relapse free survival (RFS), non-relapse mortality (NRM) and graft-versus-host disease (GVHD)-free/relapse-free survival (GRFS) since HCT was  $55.3 \pm 3.1\%$ ,  $66.0 \pm 3.2\%$ ,  $28.2 \pm 0.3\%$  and  $32.9 \pm 3.1\%$ , respectively. There was no significant difference according to the infused CD34+ cell dose (Figure1). The relapse rate was not proportionally affected by the cell dose (22.4% vs 26.7%,  $P=0.712$ ). And there was no significant correlation between the number of CD3+ and CD34+ cells infused (Spearman correlation coefficient:  $P=0.307$ ). In a univariate analysis, patients transplanted with the higher CD34+ cell doses and higher CD3+ cell doses had

no increased GRFS ( $P=0.623$  and  $P=0.158$ ). An independent factor associated with worse GRFS was risk status at transplant (HR=1.782, 95% CI:1.267–2.509,  $P=0.001$ ). These results suggest that careful assessing the CD3+ and CD34+ graft content and tailoring the cell dose infused may help in reducing cGVHD risk without negative impact on GRFS. A large and prospective study in a homogenous population will be needed to confirm the effect of stem cell dose.

**Disclosure of conflict of interest:** None.

**P195**

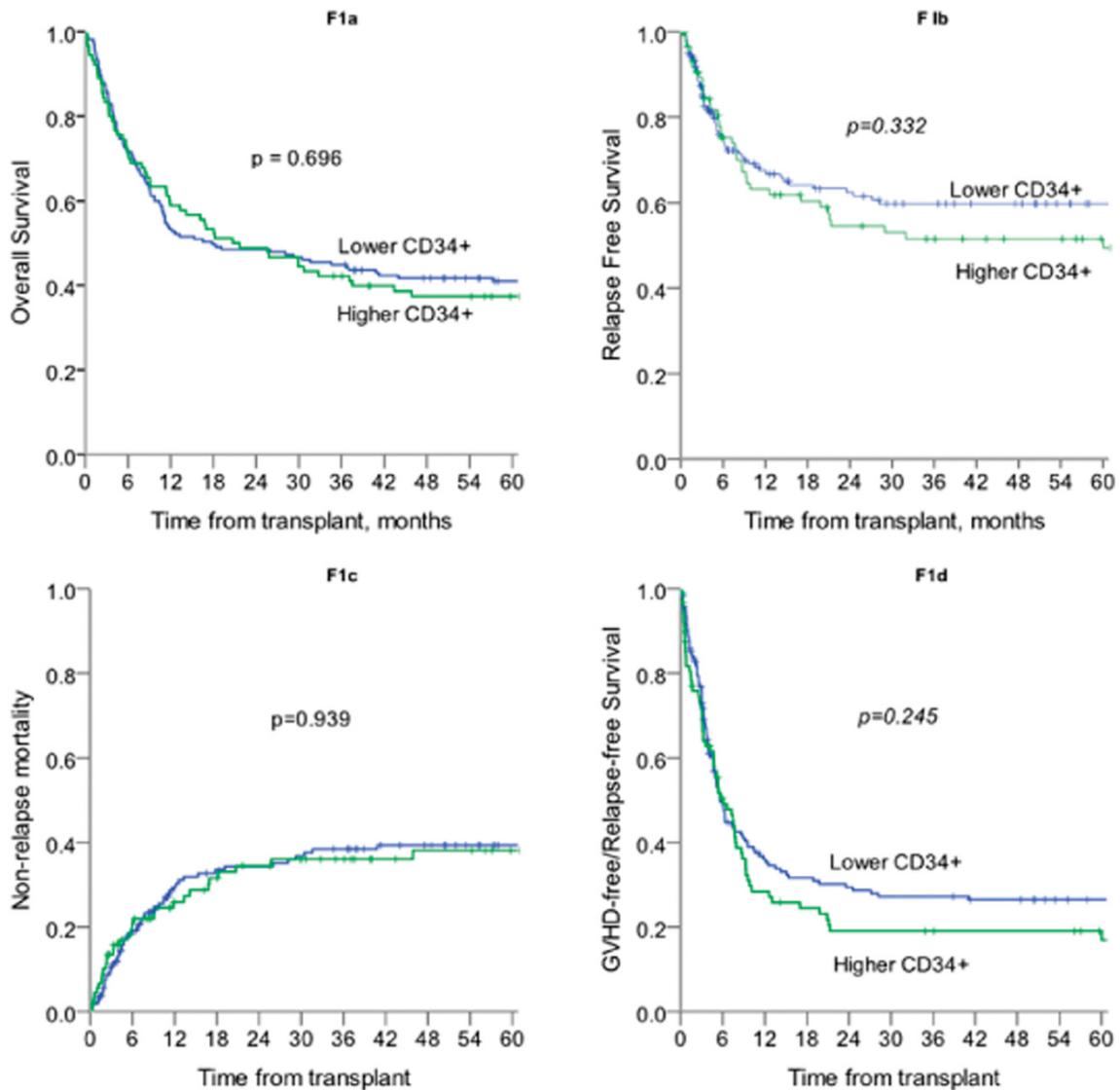
**Imatinib associated with extracorporeal photopheresis can fully reverse severe sclerotic-type lesions in patients with chronic graft-versus-host disease: the Lille University Hospital experience**

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Severe sclerotic-type chronic graft-versus-host disease (cGVHD) is difficult to reverse and can dramatically alter the quality of life of patients after allogeneic hematopoietic cell transplantation (allo-HCT). Imatinib or extracorporeal photopheresis (ECP) used separately yield sustained responses in

[P194]



**Table 1. Patients characteristics and follow-up data of seven patients receiving the combination of imatinib and extracorporeal photopheresis**

Patient #	Age	2014 NIH grade of cGVHD	Sclerotic-type	Steroid-refractory	Organs involved	Number of prior therapies	Best overall response	Time to best response (months)	Duration of combination (months)	Follow-up duration (months)	Status at last follow-up
1	48	Severe	Yes	Yes	Skin, mouth, eye	2	PR	3	18	40	Alive in PR on ECP maintenance
2	46	Severe	Yes	Yes	Skin, mouth, tendons	4	PR	3	25	25	Progression on combination
3	23	Severe	Yes	Yes	Skin, mouth, tendons	4	CR	13	36	54	Alive in CR, off combination
4	35	Severe	Yes	Yes	Skin, mouth, tendons	5	CR	8	60	78	Death in CR (myocardial infarction)
5	33	Severe	Yes	Yes	Skin, mouth, tendons	3	CR	4	4	76	Alive in CR On ECP maintenance for 47 months, currently off combination
6	57	Severe	Yes	Yes	Skin, liver	3	PR	7	32	32	Death in PR on ECP maintenance (solid tumour)
7	46	Severe	Yes	Yes	Skin	2	CR	3	43	53	Alive in CR off combination

NIH : National Health Institute ; cGVHD : chronic graft-versus-host disease ; PR : partial response ; CR : complete response ; ECP : extracorporeal photopheresis

only about 30% of patients with steroid-refractory cGVHD. Given their respective modest efficacy we hypothesized that the combination of imatinib with ECP could lead to higher response rates. We are reporting here on seven patients with severe steroid-refractory sclerotic-type cGVHD treated at our institution using this combination. We retrospectively analysed all patients treated at our institution ( $n=7$ ) with the combination of imatinib with ECP for severe steroid-refractory scGVHD. Imatinib was started at 200 mg/day and increased to 400 mg/day if well tolerated. The CELLEX closed system was used for ECP. ECP was initiated twice weekly during 4 weeks. After this « induction » period, ECP sessions were scheduled less frequently according to the response to treatment. Additional immunosuppressants were tapered gradually in responding patients. Initial grading and response evaluation was determined according to the NIH 2014 criteria. Steroid-refractoriness was defined as progression of GVHD on high-dose steroids ( $\geq 1$  mg/kg) or progression during corticosteroid tapering. Patient characteristics are displayed in Table 1. Patients received an allo-HCT between May 2004 and April 2011. Median age at allo-HCT was 47 (range: 23–57). A variety of myeloablative ( $n=2$ ) and non-myeloablative conditioning regimens were used ( $n=5$ ). Antithymocyte globulin was used before allo-HCT in one patient. GVHD prophylaxis consisted of ciclosporine and methotrexate in six patients. One patient received tacrolimus and methotrexate. Five patients had prior history of acute GVHD. NIH global severity grade was severe in all patients ( $n=7$ ) due to severe sclerotic features. The median number of previous therapies was 3 (range: 2–5). All patients were steroid-refractory. After a median follow-up of 54 months (range: 13–76 months) the overall response rate was 100%. The complete response rate was 57%. Median time on ECP associated with imatinib was 36 months (range: 4–60 months). Median time to best response was 7 months (range: 3–22 months). Corticosteroids could be discontinued in all patients after a median time of 8 months (range: 6–44 months). Patients #1, #5 and #6 received maintenance therapy with ECP upon discontinuation of imatinib. In four patients, both ECP and imatinib led to complete response and could be discontinued after 38, 74, 4 and 53 months for patients #3, #4, #5 and #7, respectively. Patient #4 and #6 passed away after due to a myocardial infarction and the development of a solid tumour, respectively. Patient #4 was off therapy while patient #6 remained on maintenance with ECP. Both remained in complete response. Patient #2 remained in response during 25 months before progression of cGVHD while on imatinib and ECP. None of our patients experienced adverse events related to either imatinib or ECP. Despite the limited number of patients in this report, we observed that the combination of

imatinib and ECP can lead to complete and sustained reversal of severe steroid-refractory sclerotic-type cGVHD. These encouraging results should be confirmed in a larger cohort.

**Disclosure of conflict of interest:** LM: Therakos (honorarium).

#### P196

##### Impact of acute graft versus host disease development on overall survival in acute myeloid leukemia

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Allogeneic hematopoietic cell transplantation (allo-HSCT) is an established treatment modality that is potentially curative for many patients (pts) with acute myeloid leukemia (AML). AML itself is the most common indication for pts undergoing HSCT nowadays. For pts with high-risk disease, allo-HSCT is, perhaps, the most effective curative treatment and is considered the standard post-remission therapy in first complete remission (first CR). This is a retrospective study to analyze those variables which were associated with patients' overall survival (OS) after allo-HSCT. The study population consisted of 31 pts who were diagnosed of AML from January 2010 to July 2016 at the Hospital Universitario Central Asturias, and submitted to allo-HSCT in first CR. Risk status based on validated cytogenetics and molecular abnormalities following recommendations of European LeukemiaNet was performed. Sixteen (51.6%) were male. Median age was 42 years old (range: 1–64). Clinical characteristics at transplantation are represented in Table 1. Median follow-up was 27 months (5–75). Considering the donor type, OS at 1 year was higher in pts receiving SD (91.8%) compared to 66% in those who received URD ( $P=0.012$ ). Regarding graft source, OS at 1 year was 88.9% who received PBSC compared to 48% in pts receiving BMSC ( $P=0.012$ ). Gender also showed significant association with OS, which was higher among men, OS at 1 year was 100%, compared to 47.4% for women ( $P=0.002$ ). The presence of minimal residual disease (MRD) detected using multiparametric flow cytometry was performed prospectively after induction and consolidation, and before transplantation. Thirteen pts had negative MRD before transplantation. Median OS was greater in pts with negative MRD before transplantation compared to the group with positive MRD (67 vs 27 months, respectively) ( $P=0.24$ ). This difference did not reach statistical significance probably because the low number

of the sample. Thirteen pts developed aGVHD. Only 4 (28.6%) pts receiving SD developed aGVHD compared to 8 (50%) pts among those who had an URD; however this association was not statistically significant ( $P=0.23$ ). Also, we observed higher incidence of aGVHD in BMSC group (6 pts; 60%) whereas only 7 (36.8%) in PBSC group developed aGVHD. This tender did not reach significant association ( $P=0.2$ ). One year OS was 59.8% in pts who developed aGVHD and 87.1% who did not ( $P=0.05$ ). All factors that had a significant influence on pts survival were included in a multivariate analysis (Cox regression model): graft source, donor type, pts gender and aGVHD development. Developing aGVHD kept an independent association with mortality (OR 6.12, 95% CI 1.39–27.29,  $P < 0.001$ ) and male gender also persisted as an independent protective factor (OR 0.12, 95% CI 0.02–0.06,  $P=0.003$ ). In our series, aGVHD has shown a significant and independent association with OS over other parameters such as graft source, type of donor or MRD before transplantation. Identifying reliable predictors for aGVHD development, controlling well known risk factors for this disease, as well as improving management of immunosuppressors should still be the key to potentiate longer OS in our patients. Larger studies are needed to confirm our results.

### References

1. Baron F, Maris MB, Sandmaier BM *et al*. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 2005; **23**: 1993–2003. [P196]

Variable	Number of patients (n=31) (%)	
Age status	Low-risk	9 (29)
	Intermediate-risk	10 (32)
	High-risk	12 (38.7)
Graft source	Bone marrow (BMSC)	10 (32.3)
	Peripheral blood (PBSC)	19 (61.3)
	Cord blood (UCSC)	2 (6)
		2 (6)
Donor type	Unrelated donor (URD)	18 (58.1)
	Matched siblings (SD)	14 (45.2)
	Haploidentical HSCT	1 (3.2)
		1 (3.2)

**Disclosure of conflict of interest:** None.

### P197

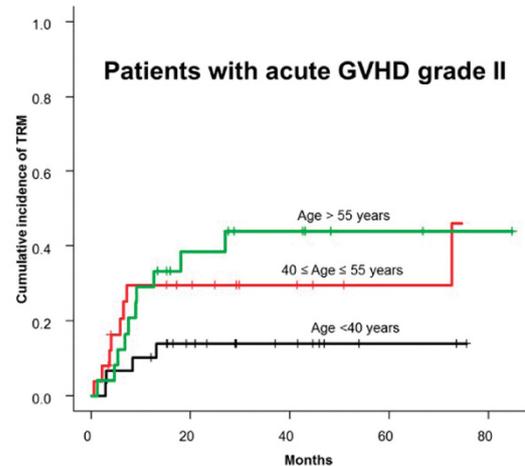
#### Impact of age on transplant-related mortality in patients with acute and/or chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation for hematological malignancies

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Acute and chronic graft-versus-host diseases (GVHD) are associated with increased morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Older patients undergoing allo-HSCT may experience a high degree of transplant-related complications and this concern has historically limited the use of allo-HSCT for some older patients. In many studies, age has been shown to be a negative prognostic factor for survival and associated with higher transplant-related mortality (TRM). However, in others, age was not shown to be a significant factor if appropriate adjustments for other comorbidities are incorporated in the analyses. There are very few studies that evaluated the relationship between patient's age, the presence of GVHD and long-term transplantation outcome. The aim of this study is to evaluate the impact of age in patients who develop acute and/or chronic GVHD after allo-HSCT for hematological malignancies on the TRM incidence. We included in the study 595 patients with hematological malignancies who received allo-HSCT and were followed in our center between January 2008 and January 2016. For the purpose of this study, only patients who developed grade II–IV acute GVHD and/or limited or extensive chronic GVHD were considered for analysis ( $N=306$ ). Patients were split into three homogeneous groups according to age at transplantation taking into

consideration the underlying disease, type of conditioning and disease response at transplantation. Group 1 (younger) included patients aged  $< 40$  years ( $N=103$ ), group 2 (intermediate) included patients aged between 40 and 55 years ( $N=102$ ) and group 3 (older) included patients older than 55 years ( $N=101$ ). GVHD evolution over time was followed as well as the cumulative incidence of TRM was calculated in case of acute or chronic GVHD in each group. Thirty seven percent of grade II GVHD occurred in the younger group ( $N=29$ ), 32% ( $N=25$ ) in the intermediate group and 31% ( $N=24$ ) in the older group; majority (69%) resolved in the younger group as well as in 24 and 46% in the latter two groups, respectively, while TRM rates at 1 year were 10%, 30% and 30%, respectively,  $sdHR=4.8$ ,  $P=0.01$ . Among patients who had acute GVHD grade II, 51, 36 and 59% in the three respective groups developed chronic GVHD later. Grade III–IV GVHD occurred in 21% ( $N=20$ ) in the younger group, 38% ( $N=38$ ) in the intermediate group and 38% ( $N=35$ ) in the older group; with a respective resolution in 20%, 26% and 17% of patients and were associated with comparable TRM rates at 1 year of 39%, 40% and 47%, respectively,  $P=0.5$ . Among patients who had acute GVHD grade III–IV, 40, 37 and 38% in the three respective groups developed chronic GVHD later. *De novo* chronic GVHD was observed with a higher rate in the intermediate and in the older group (Table) while patients with extensive chronic GVHD older than 55 years had significantly higher TRM at 2 years (47%) compared to 32% in those younger than 55 years,  $sdHR=1.9$ ,  $P=0.04$ . Patients who develop acute GVHD grade III–IV could incur over 40% of TRM at 1 year independently of age. Resolution of acute GVHD grade II was significantly better in younger patients while older patients with grade II acute GVHD or with extensive GVHD had higher mortality compared to younger ones. In addition to an adapted prophylaxis, a better preemptive GVHD strategy should be warranted in older patients.

[P197]



	Age < 40 years N=103	40 ≤ Age ≤ 55 years N=102	Age > 55 years N=101
<b>Acute GVHD gII (N)</b>	<b>29 (37%)</b>	<b>25 (32%)</b>	<b>24 (31%)</b>
- Resolutive	20 (69%)	6 (24%)	11 (46%)
- Successive cGVHD			
Limited	12 (41%)	5 (20%)	5 (21%)
Extensive	3 (10%)	4 (16%)	9 (38%)
TRM @ 1 year:	10%	30%	30%
<b>Acute GVHD gIII-IV (N)</b>	<b>20 (21%)</b>	<b>38 (41%)</b>	<b>35 (38%)</b>
- Resolutive	4 (20%)	10 (26%)	6 (17%)
- Successive cGVHD			
Limited	4 (20%)	6 (16%)	3 (9%)
Extensive	4 (20%)	8 (21%)	10 (29%)
TRM @ 1 year:	39%	40%	47%
<b>De novo Chronic GVHD (N)</b>			
Limited	16 (24%)	23 (35%)	27 (41%)
Extensive	13 (29%)	16 (36%)	15 (34%)
TRM @ 2 years:	32%	32%	47%

**Disclosure of conflict of interest:** None.

**P198**

**In vivo effects of Nilotinib on lymphocyte subpopulation and function following allogeneic stem cell transplantation**

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Chronic graft versus host disease (cGVHD) is a major complication of allogeneic stem cell transplantation and is characterized by frequent multiorgan involvement resembling autoimmune diseases; its pathogenesis is still incompletely defined and a standard treatment is lacking. Donor-derived CD4+ and CD8+ T lymphocytes have been considered the main effector cells mediating cGVHD pathogenesis; however, recent studies suggest that B cells might also play an important role. *In vitro* data indicate that tyrosine kinase inhibitors (TKIs) such as Imatinib and Nilotinib affects both innate and adaptive immune response by interacting with different cell populations (T cells, B cells, dendritic cells, mast cells and macrophages). We sought to evaluate the impact of different doses of Nilotinib on the distribution and function of lymphocyte subpopulations. We analyzed 44 samples obtained from 15 patient with steroid-dependent/refractory cGVHD enrolled in a phase 1–2 study with Nilotinib in steroid-refractory cGVHD (NCT01810718): triplets of patient were treated with escalating doses starting from

200 mg/die (5), 300 mg/die (5), up to 400 mg/die (5). Blood and plasma were collected at baseline and at day 90 and 180 of therapy. Trough plasma Nilotinib concentrations had been previously determined by HPLC (Abstract C039, Haematologica: Evaluation of Nilotinib safety in patients with steroid-refractory chronic Graft-Versus-Host Disease: a phase I-II GITMO study). Peripheral blood mononuclear cells were isolated by density gradient centrifugation using Ficoll Biocoll. Six color flow cytometry analysis (Facs Canto II) was performed using conjugated antibodies (anti-CD3, CD4, CD25, CD16, CD56, CD19). Inflammatory cytokine analysis was performed on plasma samples according to the instruction of BioPlex Pro Human Cytokine 17plex Assay (Bio-Rad). Statistical analysis was performed by 2-tailed Student's t-test; differences were considered statistically significant for  $P < 0.05$ . Flow cytometry analysis showed that Nilotinib did not exert any significant impact neither on the proportion of T lymphocytes subpopulation (CD3+CD4+ T helper, CD3+CD4+CD25+ T regulatory, CD3+CD4- T cytotoxic), nor on B lymphocytes and NK cells. On the contrary, a statistically significant and dose-independent decrease of pro-inflammatory and Th-17 cytokine production was observed (Figure 1): reduction of IL2 ( $P < 0.02$ ), IL10 ( $P < 0.05$ ) and IFN $\gamma$  ( $P < 0.02$ ) were already significant after 90 days; decreases of IL17 ( $P < 0.05$ ) and TNF $\alpha$  ( $P < 0.02$ ) become significant after 180 days. Interestingly, after 180 days of therapy, among the 21 patients enrolled (according to the ITT criteria) ten patients showed cGVHD improvement and the other five remained stable. This study shows that therapeutic doses of Nilotinib can reduce plasma levels of inflammatory cytokines without affecting the proportions of lymphocyte subpopulations. These findings correlate with clinical response and suggest that besides the previously demonstrated anti-fibrotic effects, Nilotinib has also potent anti-inflammatory and immune regulatory properties, supporting its role in patients with cGVHD. **Disclosure of conflict of interest:** None.

[P198]

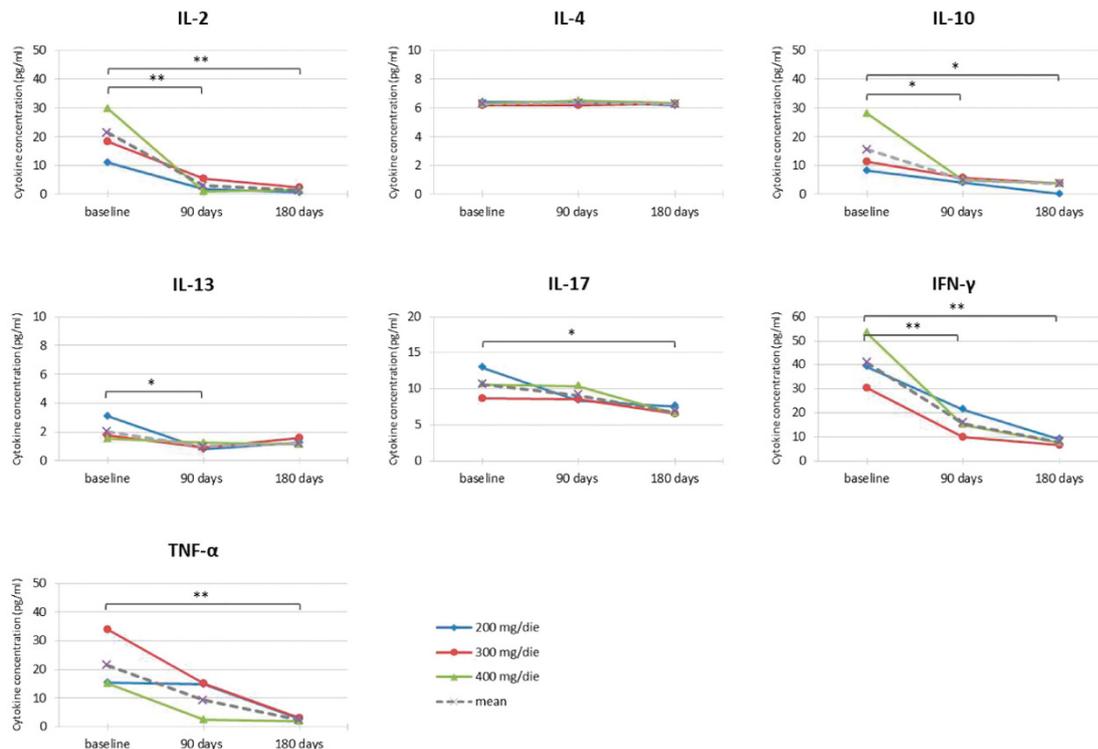


Figure 1: We analyzed 44 samples obtained from 15 patient with steroid-dependent/refractory cGVHD enrolled in a phase 1-2 study with Nilotinib in steroid-refractory cGVHD (NCT01810718): triplets of patient were treated with escalating doses starting from 200 mg/die (5), 300 mg/die (5), up to 400 mg/die (5). Blood and plasma were collected at baseline and at day 90 and 180 of therapy. Inflammatory cytokine analysis was performed on plasma samples according to the instruction of BioPlex Pro Human Cytokine 17plex Assay (BioRad). Statistical analysis was performed by 2-tailed Student's t-test; differences were considered statistically significant for  $p < 0.05$ . (\*  $p < 0,05$  \*\*  $p < 0,02$ )

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Previously published

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**Infectious gastro-enteritis after allogeneic hematopoietic transplantation after reduced intensity conditioning (allo-RIC): incidence and possible role in gastro-intestinal acute GVHD**

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Enterotoxigenic *C. difficile*-associated disease or infection (CDI) is a common cause of diarrhea after hematopoietic stem cell transplantation (SCT). Recent studies have suggested the relationship of CDI with gastro-intestinal (GI) graft-versus-host disease (GVHD). The possible role of other types of infectious gastro-enterocolitis (G-EC) in GVHD development has not been studied. As a prior investigation to a national prospective observational study on this issue, we conducted a single-center retrospective analysis including all adult patients who received an allo-RIC SCT between January 2010 and March 2016. The aim was describing the cause(s) (if known), timing and outcomes of recipients with possible G-EC (defined as new onset acute diarrhea grade  $\geq 2$ ) in the first year after SCT. Of the 123 patients studied (median age: 54 years, 62% male, 49% AML or MDS as underlying disease), 97 (79%) had a total of 148 episodes of acute diarrhea, with 35 (28%) developing more than one event. These acute diarrheas occurred at a median of 39 days (range: 1–363) after SCT. Overall, a G-EC causing pathogen was identified in 33 of 148 stool specimens (22%) and included: CDI (7), *C. jejuni* (7), rotavirus (7), adenovirus (2), norovirus (2), *B. hominis* (3), *S. stercoralis*, *G. lamblia*, *A. caviae*, *Salmonella enterica* and cryptococcus (one in each case). Most posttransplant diarrheas (68/148; 46%) occurred during the 4 weeks after infusion and were attributable to mucosal damage caused by the RIC (negative microbial screening and no evidence of GVHD). The rate of infectious G-EC among the diarrheas occurring after day +30 was 41% (33/80). The overall incidences of enteric infection were 12.7% (95% CI: 6.5–18.9) and 17.6% (95% CI: 10.4–24.8) at +6 and +12 months after SCT, respectively. All the infected patients had mild to moderate disease, and no deaths were attributable to this complication. There were no differences in 2 year-OS and NRM between the infected and uninfected patients (81% vs 73%,  $P=0.6$  and 16% vs 19%,  $P=0.7$ , respectively). In univariate analysis age < 50 years, prior SCT, donor type, ATG administration and prior grade 2–4 aGVHD were associated with development of infectious gastro-enteritis. In multivariate analysis, unrelated donor and grade 2–4 aGVHD were the only factors significantly associated with gastrointestinal infection (HR 2.7; 95% CI: 1.1–6.5,  $P=0.02$  and HR 3.6, 95% CI 1.5–8.5,  $P=0.004$ ; respectively). Acute GVHD occurred in 46% of patients ( $n=56$ ), with a median onset of 54 days (range: 4–231). The cumulative incidences of 2–4 acute GVHD at 100 days and 6 months post-SCT were 21% (95% CI: 15.3–32%) and 32.6% (95% CI: 23.4–42%), respectively, and there was a trend toward a higher risk of 2–4 GVHD in the group of patients with an enteric pathogen (48.2% vs 27% at 1 year,  $P=0.06$ ). More importantly, an enteric infection occurred just before the onset or aggravation of GVHD in 12/33 infected patients in our study (36%) at a median interval of 8 days after the infection (range: 0–24). In summary, our results confirm that enteric infections are a common complication after Allo-RIC, representing at least 20% of the episodes of acute diarrhea during the first year post-SCT. A possible interplay between infectious G-EC and GVHD was observed in this study.

**Disclosure of conflict of interest:** None.

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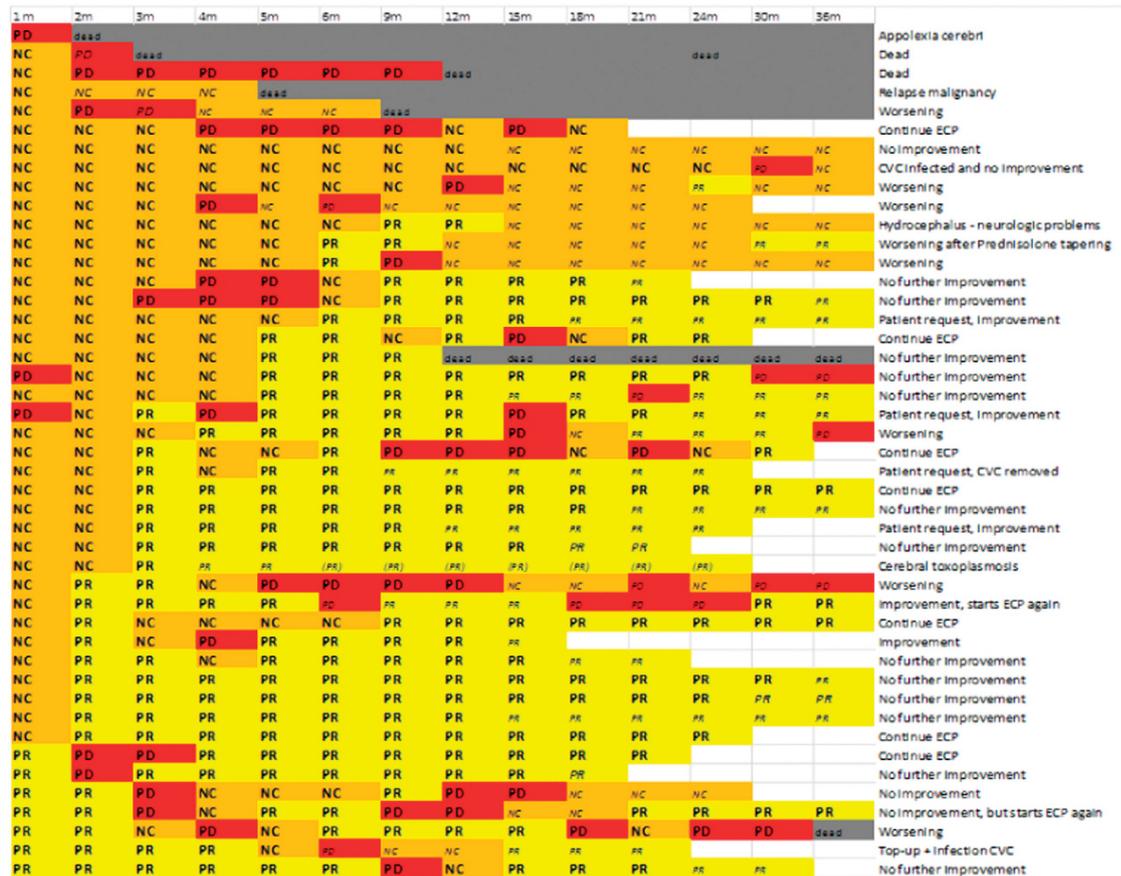
**Long-term efficacy of extracorporeal photopheresis in chronic graft versus host disease**

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Chronic graft versus host disease (cGVHD) activity is known to fluctuate over time, so we evaluated cGVHD continuously throughout the Extracorporeal Photopheresis (ECP) treatment course and after stopping ECP. Patients with at least 1 year follow-up, who were treated with ECP at Department of Dermatology, Bispebjerg hospital between 2009 and 2015 were evaluated. A single investigator retrospectively evaluated response to ECP monthly for 6 months, every 3 months until 2 years and every 6 months until 3 years. Prednisolone doses were recorded every 3 months. Responses were defined as complete remission (CR) if no symptoms of cGVHD were present, partial remission (PR) as improvement in cGVHD or stationary cGVHD with more than 50% reduction in Prednisolone, no change (NC) as no difference in symptom burden and < 50% reduction in prednisolone. Progressive disease (PD) was defined as worsening of symptoms with unchanged or intensified immunosuppressive medication. ECP was performed with Therakos UVAR XTS or Cellex. There were 45 evaluable patients with moderate ( $n=20$ ) or severe ( $n=25$ ) steroid-refractory, dependent or -intolerant cGVHD. The median age was 58 years (range: 19–71) and there were 22 females and 23 males. Conditioning regimen was myeloablative ( $n=10$ ) and non-myeloablative ( $n=35$ ). Seventeen had related donors and 28 had unrelated. Stem cell source was peripheral blood ( $n=36$ ), bone marrow ( $n=7$ ) or umbilical cord blood ( $n=2$ ). Number of organs affected by cGVHD was one ( $n=8$ ), two ( $n=16$ ), three ( $n=11$ ), four ( $n=9$ ) or five ( $n=1$ ) and involved organs were skin ( $n=36$ ), eyes ( $n=26$ ), mouth ( $n=24$ ), lungs ( $n=8$ ), genitals ( $n=6$ ), liver ( $n=6$ ), musculoskeletal system ( $n=5$ ) or gastrointestinal tract ( $n=2$ ). Time from diagnosis of cGVHD to first ECP was median 444 days (range: 11–2760) and time from referral to ECP and the first ECP procedure was median 52 days (range: 11–178). At the time of the first ECP procedure patients were also treated with prednisolone ( $n=43$ ), sirolimus ( $n=21$ ), calcineurin inhibitor ( $n=18$ ), mycophenolate mofetil ( $n=5$ ), imatinib ( $n=4$ ), methotrexate ( $n=1$ ) or rituximab ( $n=1$ ). One patient received no immunosuppression. Total number of ECP cycles was median 20 (range: 1–61). Responses over time are shown in Figure 1. Overall response to ECP was seen in 25 (56%) of the patients. Most responses were seen after more than 3 months ECP treatment. In univariate analysis of possible baseline predictors of response, no significant associations were found. Prednisolone dose was significantly reduced at every 3 months after start of ECP ( $P<0.01$ ). Additional cGVHD treatment was administered to 14 (31%) patients during ECP treatment (sirolimus  $n=6$ , calcineurin inhibitor  $n=6$ , UVA1  $n=4$ , methotrexate  $n=3$ , rituximab  $n=3$ , mycophenolate mofetil  $n=2$ ). About 6 (13%) patients had more than 1 additional treatment. Prednisolone dose was increased at least once in 20 (44%) patients during ECP treatment. Overall survival at 5 years was 80%. Follow up was median 694 days (range: 62–2416). More than half the patients with cGVHD (56%) improve overall after treatment with ECP, but flares in cGVHD activity still occur. Prednisolone dose is significantly reduced at all time points after starting ECP, but short term increased doses or additional immunosuppression was necessary in more than one-third of the patients. Larger prospective studies with long-term end points are warranted.

**Disclosure of conflict of interest:** Marietta Nygaard has received a travel grant and speaker's fee from Therakos/Malinckrodt.

[P201]



**Figure 1** The patients' treatment courses are shown horizontally and on the right are the reasons for stopping ECP. GVHD activity is colourcoded: Orange = No change; Red = Progressive disease; Yellow = Partial remission; Grey = dead; White = timepoint not yet reached. **Bold** letters indicate the patient is still in active ECP treatment. *Italic* letters indicate ECP has stopped.

**P202**  
**Long-term outcome of NIH response criteria in 28 patients receiving imatinib for refractory chronic GVHD**

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Chronic graft versus host disease (cGVHD) remains a major cause of morbidity and mortality after hematopoietic stem cells transplantation despite the improvement of the immunosuppressive prophylaxis. Skin, buccal, lacrimal and hepatic disorders are the most frequent. Sclerotic GVHD remains a severe form and often refractory to standard treatment lines such as corticosteroids and calcineurin inhibitors. The antifibrotic activity of Imatinib by the inhibition of PDGF-R and TGFβ pathways has been used in the treatment of refractory GVHD with sclerotic features and systemic scleroderma. Here, we report the results of Imatinib treatment in 28 patients (pts) with refractory cGVHD. Over a period of 13 years (January 2000–December 2012), 1308 pts received allogeneic stem cells transplantation from related donors, 28 of whom were treated with Imatinib for refractory cGVHD: 24 pts for malignant diseases (14 CML, 9 AML, 1 NHL) and 4 pts for aplastic anemia. The median age is 31 years (6–55), the sex ratio M/F: 2.1. Conditioning regimen used with chemotherapy alone: myeloablative (14 pts with GVHD prophylaxis combining ciclosporin and methotrexate), reduced intensity (14 pts with prophylaxis combining ciclosporin-mycophenolate mofetil).

All pts received peripheral blood stem cell transplant with an average of CD34 cell count:  $7.1 \times 10^6/\text{kg}$  (1.23–21.8). The median duration of the cGVHD is 8 months (3–27). The first-line treatment consisted of the combination of steroids-ciclosporin with or without mycophenolate mofetil. Imatinib was administered to these pts after median treatment duration of 48 months (9–108) for moderate (2 pts) and severe (26 pts) cGVHD according to the NIH classification. Treatment with Imatinib, at doses ranging from 100 to 400 mg/d, was introduced in the second line for all pts. The evaluation is conducted in October 2016 after a median follow-up of 128 months (76–189). Tolerance was good except in a one pt with severe thrombocytopenia that led to a transient cessation of treatment. After 6 months, analysis of pts who received Imatinib according to Couriel criteria and NIH criteria: complete remission (CR): 1 pt (18%), partial remission (PR): 20 pts (71%), stable disease (SD): 5 pts, failure: 2 pts (7%). A long-term evaluation performed after a mean duration of treatment 62 months (6–91) finds similar results with a CR: 4 pts (14%), PR: 18 pts (64%), SD: 2 pts (7%) and failure: 4 pts (14%). Corticosteroids were tapered or discontinued in 12 pts (CR or PR). At October 2016, 26 pts (93%) were alive and 2 pts (7%) died of severe infections. Treatment with Imatinib seems to be a good therapeutic option in the treatment of cGVHD in its moderate or severe form refractory to a minimum of two immunosuppressive agents according to the NIH criteria as shown by our results in terms of response and survival with good tolerance.

**Disclosure of conflict of interest:** None.

**P203**

**Low early doses of ATG are effective in GVHD prevention in unrelated myeloablative stem cell transplants for acute leukemia and myelodysplasia**

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ATG significantly reduces the risk of cGVHD both in unrelated and in HLA identical sibling. The Finke's study<sup>1</sup> randomised pts undergoing an allogeneic unrelated stem cell transplant (SCT) after a myeloablative regimen to receive or not 60 mg/kg ATG-Grafalon reporting a significant reduction of cGVHD without increase of relapse and no OS and DFS effect. However a successive study<sup>2</sup> didn't confirm those results (significant reduction of acute and chronic GVHD but poorer survival mainly due to higher relapse probability in the ATG arm). The conflicting data reported on URD SCT have several explanations, one is about the dose and the timing of ATG. The timing of ATG infusion has been demonstrated to be crucial for CB transplant<sup>3</sup>: an earlier administration is still active in preventing GVHD while ensuring engraftment and low hampering of immune reconstitution. Here we report a large (193 SCT) retrospective monocentric analysis on low ATG doses (and 15–25 mg/kg for BM according to the degree of HLA matching and 30 mg/kg for all PBSC SCT) given early (from day -6 to -2). Pts in the study were AML (n=112, 58%), ALL (n=57, 30%), HR MDS (n=21, 11%), CR1 (n=111, 66%) CR2 or >(n=31, 18%), active disease (n=27, 16%) for AL; median age was 46 (range: 18–66). Myeloablative conditioning were BU-Cy120 (n=72, 37%), Bu-Flu (n=61, 32%), Edx-TBI (n=20, 10%), other (n=40, 21%); PBSC was used in 41% (n=80); SCT were performed between 2005 and 2015 at the Bologna Transplant Center. SCT were performed from HLA 10/10 identical URD (n=63, 33%), or from 9/10 (n=93, 48%), 8/10 (n=30, 16%) and <8/10 (n=7, 4%). Median follow up was 55 months. Overall, grade 2–4 aGVHD was 26%, grade 3–4 aGVHD 9%; cumulative incidence (CI) of cGVHD of any severity was 25%, for moderate–severe cGVHD 18%. CI of relapse and NRM was 28% and 21%, respectively. The 3-year overall and disease-free survival were 60% (95% CI: 52–67%) and 60% (95% CI: 51–68%). The GVHD (aGVHD grade 3–4 and moderate–severe cGVHD) and relapse free survival (GRFS) of the entire population (Figure 1) was 45% at 3 years (95% CI: 37–52%). Restricting the analysis to patients in CR1–2, we found that cGVHD (any severity), GFRS and OS at 3 years were 23%, 50% and 64%, respectively. Comparing transplants with 10/10 URD to mismatched ones (9/10 or less)

we found a trend for increased mod/sev cGVHD in pts undergoing transplant with mismatched URD (SHR 2.32, 95% CI: 0.95–5.65, P=0.06); aGVHD grade 3–4 and cGVHD overall were not significantly increased; relapse incidence according to HLA mismatches resulted 33% and 26% in 10/10 and ≤9/10, respectively; GRFS was 52% in 10/10 and 41% in ≤9/10. The data reported show that low and early administration of ATG is able to effectively prevent acute and chronic GVHD without increasing relapse thus ensuring really convincing GRFS, even for <10/10 matched URD transplants.

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**Disclosure of conflict of interest:** None.

**P204**

**Mesenchymal stem cells for the treatment of steroid refractory graft versus host disease after allogeneic stem cell transplantation**

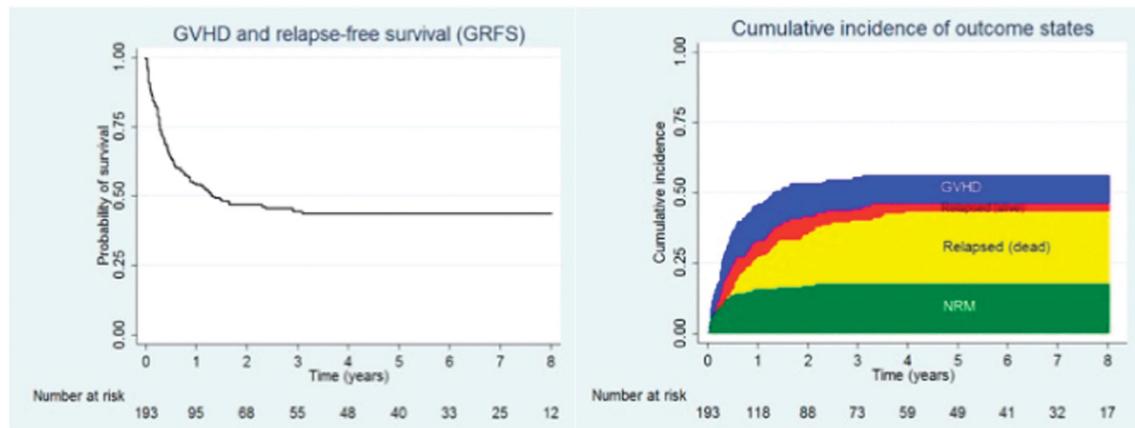
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Graft versus host disease (GVHD) is one of serious complications in patients after allogeneic hematopoietic stem cell transplantation. The application of mesenchymal stem cells (MSC) represents a promising method for the treatment of severe steroid refractory GVHD. We present the data from an interim analysis of clinical trial, within which we applied MSC in 28 patients with acute or chronic GVHD after allogeneic transplantation. The diagnoses included AML (12 pts), MDS (5 pts), ALL (2 pts), CLL/NHL (5 pts), MPN (4 pts). The patients underwent sibling HLA-compatible (7), haploidentical (4), unrelated HLA-compatible (13) or HLA-mismatched (4) transplants. The median interval between the transplantation and MSC was 6 months (1–95). The indications for MSC infusion were steroid resistant acute GVHD (11 pts), steroid-dependent GVHD (aGVHD 3 pts, cGVHD 11 pts) or chronic GVHD with the need for long-term immunosuppression and corticosteroid intolerance (3 pts). MSC were applied as a single infusion at a median dose of 3.45 (0.9–5.0) × 10<sup>6</sup>/kg. Response to treatment was assessed on day 14, 30, 60 and 100. The severity of GVHD prior to MSC was graded as clinical stage 3 (2–3) in acute and stage 2 (1–3) in chronic GVHD, respectively. The median dose of corticosteroids was 0.92 (0.3–1.2) mg/kg/day in aGVHD and

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**FIGURE 1**



0.25 (0.1–0.5) mg/kg/day in cGVHD patients. On day +14 the partial response (PR) was achieved in 85% of patients with aGVHD, the stabilisation of GVHD (SD) was found in 85% of patients with cGVHD. The dose of corticosteroids was reduced in most patients with aGVHD (to 62% of the starting dose; 30–97%), while the early reduction was possible only in 36% of cGVHD patients. On day+100 only 19 patients were evaluable. The aGVHD patients (7 pts) achieved a significant clinical response: 4 PR, CR 3 and dose of corticosteroids was reduced in all of them (to 17%; 10–60%). The minor responses were achieved in cGVHD patient (12 pts.) with 3 PR and 9 SD. However the dose of corticosteroid was reduced in 83% of these cases (to 56% of the initial dose; 21–71%). A total of 10 patients died because of infectious complications. Most of them (8/10) were aGVHD patients who expired early up to day +60. There were observed no side effects of MSC application neither during the infusion nor later during the follow-up of 16 (2–45) months. The analysis of lymphocyte reconstitution revealed the changes of kinetics of some subsets as compared to the day +0 benchmark. The B-lymphocyte count tended to decrease in 82% of patients from chronic GVHD subgroup (vs 60% in aGVHD). Conversely NK cells declined in most aGVHD patients (80% vs 36% in cGVHD). Also the pro-inflammatory Th17 cell was affected especially in aGVHD (decrease in 63% pts vs 50% pts in cGVHD). The counts of myeloid/plasmacytoid dendritic cell increased in 80%/80% aGVHD and 50%/91% cGVHD patients. The screening testing of cytokines (RayBiotech, 42 cytokines, 6 pts, day +0 to +60) revealed changes of some analytes after MSC infusion, including a decrease of pro-inflammatory cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , IL-6. Our experience with the treatment of GVHD using MSC confirmed the safety of this immunotherapy. The favourable clinical effect with reduction of severity of GVHD and steroids dose was observed, especially in patients with acute form of GVHD.

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**Disclosure of conflict of interest:** None. The work was supported by grants FNPL-00669806 and 15-30661A.

#### P205

##### Methotrexate day +1 omission is not associated with higher incidence of acute graft-versus-host-disease

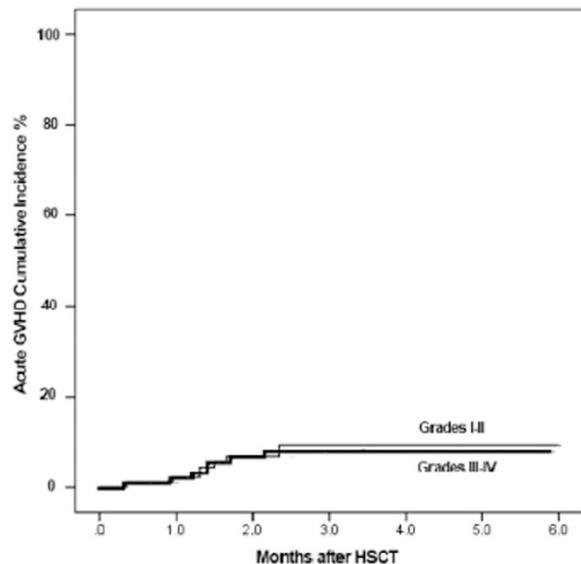
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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a high-risk procedure due to its related morbidity, limiting the broader application of this important treatment modality. Despite extensive research over the years, acute graft-versus-host-disease (aGVHD) affects the majority of patients undergoing allo-HSCT, and up to 50% will develop clinically significant grades (II). Over the years, several methods for GVHD prophylaxis have been implemented, including immunosuppressive agents. Methotrexate (MTX) is one of the earliest drugs used for GVHD prophylaxis. Frequently, a short course of intravenous methotrexate (given on days +1, +3, +6 and +11 after HSCT) is combined with a 6-month tapered course of cyclosporine. There is no consensus on which drugs and schedules for prevention of GVHD are best and clinical practice varies by institution. Further, it is not clear whether omission of the day +1 dose of MTX has a negative effect on outcome in terms of morbidity. To describe the frequency of acute and chronic GVHD, mucositis and engraftment in patients receiving methotrexate (plus CSA) as prophylaxis, omitting day +1. Ninety-five consecutive patients who underwent allo-HSCT from 1999 to September 2016, and received MTX as immunosuppressive prophylaxis were included. All patients received three doses of MTX, always excluding day +1. MTX was administered IV, either 10 mg/m<sup>2</sup> day +3, +6, +11 or 15 mg/m<sup>2</sup> day +3, and 10 mg/m<sup>2</sup> during days +6 and +11. We included 95 patients (55% male). The most frequent underlying diseases were aplastic anemia (21%) and acute lymphocytic leukemia (21%). Ninety-nine percent of patients

had a matched related donor. Forty patients (42%) had gender disparity with their donor, and 13% presented ABO incompatibility (major in 75%). Most of the patients received myeloablative conditioning regimens ( $n=73$ , 77%). The median of CD34+ infused cells were  $2 \times 10^6$  (range: 0.8–6.8). The median neutrophil and platelet engraftment was 20 (11–43) and 15 (range: 5–46) days, respectively. From all the cohort, only 15 patients (16%) developed acute GVHD (53% grades I–II) (Figure 1). Thirty patients (32%) developed chronic GVHD, which was limited in 73%. Most of the patients, 83% ( $n=79$ ), presented acute toxicity after the conditioning regimen, from which 76% ( $n=60$ ) corresponded to superior mucositis (50% grade I–II and 50% grade III–IV). The 10-year overall survival was 59% and the 10-year relapse free survival was 68%. Our results showed a low incidence of acute GVHD, mostly grades I–II, and similar survivals compared to previously reported studies, proposing that the administration of day +1 MTX as GVHD prophylaxis is not mandatory, however, prospective studies might be necessary to test our results.

[P205]



**Disclosure of conflict of interest:** None.

#### P206

##### Outcome of refractory graft versus host disease (GVHD) treated with extracorporeal photopheresis (ECP) as second line: a single-center experience

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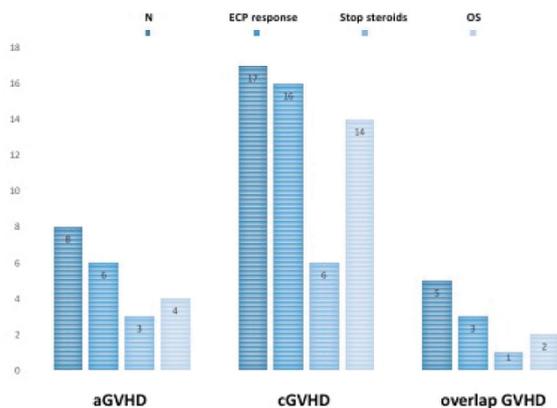
GVHD is a common and, sometimes, life-threatening entity related to hematopoietic stem cell transplantation (HSCT). Steroids remain the first-line therapy but they are not always enough to control it, or their side effects are simply unacceptable. Both acute and chronic GVHD are responsible of impairment occurred in different organs that can lead to increase morbidity and mortality in our patients. Different options are available as second line, but it is a well known fact that ECP, due to its immunomodulatory mechanism, yields satisfactory response rates and presents excellent safety profile. From May 2012 to October 2016, 30 patients with steroid-dependent or refractory GVHD have been treated in our centre with ECP. We have performed 305 ECP procedures with the Therakos Cellex device, an integrated 'on line' system. The transplant was from a sibling donor in 13 cases and 17 from an unrelated donor. The median of CD3+ infused was  $247.35 \times 10^6$  CD3/kg. Eight patients (16.7%) presented aGVHD, 17 (56.7%) cGVHD and finally, 5 (16.7%) had

an overlap GVHD syndrome. Most of patients (87.5%) with aGVHD had a severe intestinal involvement as the main manifestation of the disease. However, all patients with cGVHD had a multiorgan involvement with a median of four organs affected, being skin, mouth, eyes and lungs the most common implicated. Ten patients in our series have died, 7 for GVHD complications or infections and 3 due to relapse of AML. As first-line treatment they all received steroids and cyclosporine or mycophenolate mofetil. Median ECP per patient has been 18 (2–31). ECP procedures were performed for 2 consecutive days, in initial phase weekly (in those with aGVHD), or every 2 weeks (cGVHD) and then monthly according to clinical response, evaluated by clinical assessment and reduction in immunosuppression. About 75% of patients with aGVHD had a significant clinical response to ECP so that steroid doses could be tapered and even in 37.5% of them withdrawal was possible. In the cGVHD group overall response rate (ORR) to ECP was 94.1%. In 35% of these patients steroids could be suspended after a median of 8.5 ECP procedures. All patients who responded to ECP in cGVHD are still alive. Independently of GVHD type, 81.4% of patients responded to ECP and 37% of them even could stop steroid therapy. Those who had no response are dead. In cGVHD, 82.35% of patients remain alive, in contrast with aGVHD or overlap syndrome patients whom survival is around 40%. About adverse events, 60% of patients did not present any complication associated with ECP. Complications were mostly related to central venous catheter, with 12 cases of bacteremia and 2 thrombosis, easily recovered. In our experience, ECP is effective as second line treatment in GVHD, obtaining the best results in the chronic GVHD group. In fact, cGVHD patients with a good clinical response to ECP, specially when steroid doses can be tapered, have the better outcomes and longer survival. The tolerance to the procedure is excellent without severe adverse events. More experience is required to determine the best scheme of ECP and its role as prophylactic treatment.

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[P206]



Disclosure of conflict of interest: None.

#### P207

##### Outcomes of co-transplantation of mesenchymal stem cells and hematopoietic stem cells compared to hematopoietic stem cell transplantation alone in $\beta$ -thalassemia patients

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Mesenchymal stromal cells (MSCs) possess immunomodulatory properties and may play important roles in graft-versus-host disease (GvHD) and engraftment. This study examined co-transplantation of MSCs and HSCs (hematopoietic stem cells). We investigated co-administration of *ex vivo* expanded MSCs along with HLA-identical sibling-matched HSCs in  $\beta$  thalassemia major patients. We recruited 70 patients from January 2010 to January 2015 in our study. All participants received Cyclophosphamide-based or Fludarabine-based conditioning regimens and short-course Methotrexate and Cyclosporine as GVHD prophylaxis. MSCs were administered intravenously ( $1.0\text{--}2.0 \times 10^6/\text{kg}$ ) into patients ( $n=41$ ) 4 h before infusion of HSCs. The outcomes were then compared to those of 29 patients transplanted with HSCs alone. The median follow-up in the MSC and non-MSC group was 2.98 and 2.62 years, respectively. Median time to WBC engraftment  $> 0.5 \times 10^9/\text{L}$  was 17.7 days (range: 15–20 days) in both groups ( $P$ -value=0.83) and median time to platelet engraftment  $> 20 \times 10^9/\text{L}$  was 27.2 days (range: 22–31 days) in the MSC group, while it was 36.6 days (range: 22–50 days) in the non-MSC group ( $P$ -value=0.26). Fifty-six percent of patients had acute GVHD in the MSC group compared to the non-MSC group where 65.5% developed acute GVHD ( $P$ -value=0.42). Meanwhile, chronic GVHD was 21% in the MSC group and 37% in the Non-MSC group ( $P$ -value=0.14). Although the incidence of acute and chronic GvHD was lower in co-transplantation of HSCs and MSCs, no statistically significant difference was noted between the two groups. Three-year overall survival rate was 70% and 61% in the MSC and NON-MSC group, respectively ( $P$ -value=0.78). Three-year thalassemia-free survival rate was 54% in the MSC group and 61% in the non-MSCs group, showing no statistically significant difference ( $P$ -value=0.35). The 3-year rejection incidence in the MSC and non-MSC group was 19% and 3%, respectively ( $P$ -value=0.07). There was no statistically significant difference between the two groups in terms of 3-year transplant-related mortality ( $P$ -value=0.79). This study indicates that co-transplantation of HLA-identical sibling HSCs with MSCs does not inflict harm on bone marrow transplantation procedure and seems to be safe and secure. On the other hand, differences between the two groups in acute and chronic GVHD, engraftment, overall survival, thalassemia-free survival and rejection incidence did not reach statistical significance. Therefore, despite the immunomodulatory activity of MSCs and their role in GVHD amelioration and engraftment improvement resulted from *in vitro* studies, their efficacy in the clinical setting has not been conclusively proven which indicates further multicenter randomized clinical trials are required.

**Keywords:**  $\beta$ -thalassemia major, co-transplantation of mesenchymal and hematopoietic stem cells, engraftment, graft-versus-host disease. Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran.

**Disclosure of conflict of interest:** None.

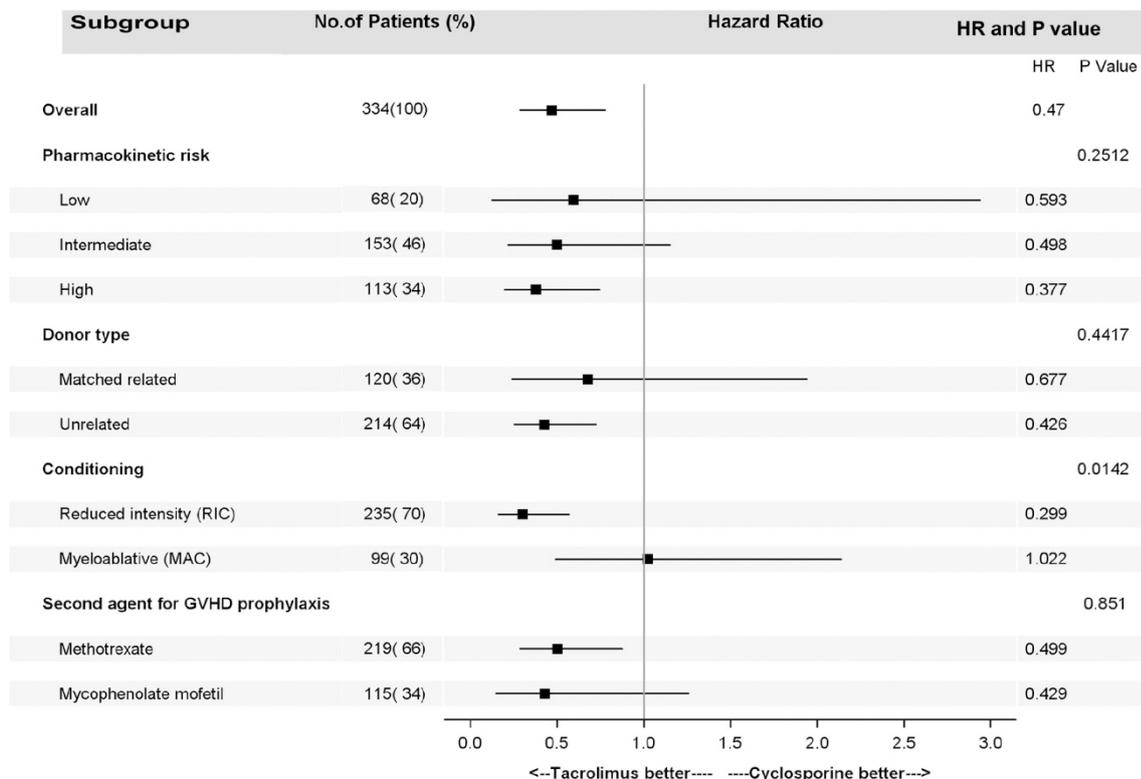
#### P208

##### Pharmacokinetic comparison of cyclosporin A and tacrolimus in graft-versus-host disease prophylaxis

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A number of studies were published with contradictory results comparing tacrolimus (Tac) and cyclosporine A (CsA) for graft-versus-host disease (GVHD) prophylaxis, but there are only few that accounted for pharmacokinetic (PK) parameters. In this retrospective study we have identified PK parameters that affected GVHD incidence and incorporated them in the



multivariate comparison of Tac- and CsA-based prophylaxis. The retrospective study included 95 consecutive patients with CsA and 239 consecutive patients with Tac prophylaxis. 36% were grafted from matched related donor (MRD) and 64% from unrelated donor (UD). About 30% received busulfan-based myeloablative conditioning (MAC) and 70% reduced-intensity conditioning (RIC). Second agent for GVHD prophylaxis was short-course methotrexate (MTX) 10–15 mg/m<sup>2</sup> on days +1, 3, 6, 11 in 66% of patients and mycophenolate mofetil 30 mg/kg days –1 to +30 in 34%. Unrelated graft recipients also received antithymocyte globulin (ATGAM, Pfizer, NY, USA) 60 mg/kg. The PK parameters analyzed were mean and median concentrations, PK variability parameters and number of concentrations below the targeted limit (NLow) within 21, 30 and 50 days after HSCT. For Tac the highest predictive value for acute GVHD was observed for median concentration during first 21 days (AUC=0.575), and for absolute skewness (AUC=0.567) of concentration data. For CsA parameters with highest predictive value were median concentration (AUC=0.547) and variability coefficient (AUC=0.736) during first 30 days. Nlow was also a significant parameter for Tac (P=0.036) and CsA (P=0.019). The model with these variables distinguished patients with low, intermediate and high risk of acute GVHD (HR 1.77, 95% CI 1.36–2.32, P=0.05) The subgroups that had benefit from Tac were second-agent Mtx patients (HR=0.499, 95% CI 0.284–0.876, P=0.015), patients with RIC (HR=0.299, 95% CI 0.157–0.571, P=0.0003) and unrelated grafts (HR=0.426, 95% CI 0.249–0.728, P=0.002) (Figure 1). Patients in Tac group had lower non-relapse mortality (HR=0.574, P=0.01) and higher overall survival (HR=0.663, P=0.03). PK risk should be accounted for in comparisons of GVHD prophylaxis regimens with calcineurin inhibitors, and Tac was superior to CsA in patients with high, but not intermediate and low PK risk.

**Disclosure of conflict of interest:** None.

**P209 Post-transplant cyclophosphamide and bortezomib vs calcineurin inhibitor-based GvHD prevention: comparison of two quasi-contemporaneous matched group**

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Current GvHD prevention regimens are partially effective, delay immune reconstitution, impair graft versus tumor effect and are cumbersome to use. Therefore, there is a pressing need to develop innovative approaches for the prevention of GvHD. We completed a phase I-II study employing a calcineurin and mTOR inhibitor-free regimen based on a combination of post-transplant cyclophosphamide and bortezomib (CyBor) in patients receiving fludarabine and busulfan-based reduced-intensity conditioning followed by peripheral blood, matched related or unrelated transplant. Patients receiving grafts from unrelated donors also received rATG. We reported that the regimen was feasible and safe and yielded promising outcomes. (1,2) Herein, we compare the results to those of a quasi-contemporaneous matched group of patients receiving a calcineurin-based GvHD prophylaxis. The experimental and control groups were well-matched in terms of age, sex, donor type, disease status, renal function and PAM score. The CyBor group (n=28) was treated during a timeframe spanning from 2012 to 2016 and the control group (n=15) from 2013 to 2016. GvHD prophylaxis for the control group was MMF and CSA (n=2) or tacrolimus (n=13). Both groups received supportive care according to standard institutional protocols. Median follow-up for the CyBor group was 28.7 months as opposed to 11.2 for the control group. Median times to neutrophil engraftment for the CyBor and control groups were 16 days (13–23) and 12 (10–21), respectively (P=0.001). Two patients from the CyBor arm died before achieving platelet engraftment. For the remaining

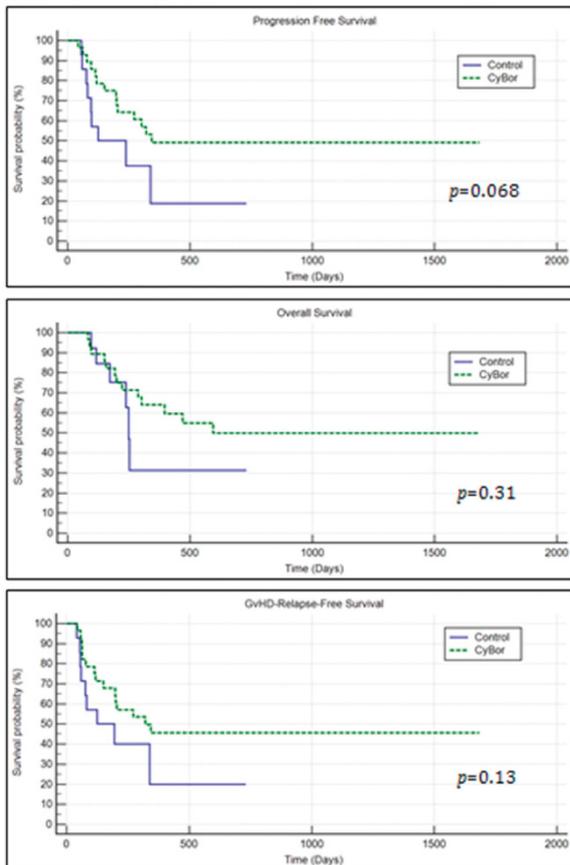
patients, median time to platelet engraftment was 27 days (15–38). For the control group, five patients never dropped their platelet count below  $20 \times 10^9/L$ . For the remaining patients, median time to platelet engraftment was 17 days (10–29) ( $P=0.002$ ). There was no primary or secondary graft failure in either of the two groups. The incidences of acute grade II–IV and grade III–IV for the CyBor group were 35.7 and 10.7%. For the control group, the incidences were 60 ( $P=0.12$ ) and 20% ( $P=0.4$ ). The incidence of chronic GvHD for the CyBor and control groups were 28% and 14.3%, respectively ( $P=0.62$ ). Treatment-related mortality was 14.3% and 20% for the CyBor and control groups, respectively ( $P=0.13$ ). The incidences of CMV, EBV and BK reactivation for the CyBor group were 57.1%, 32.1% and 17.9%, respectively. For the control group, the incidences were 46.7% ( $P=0.49$ ), 26.7% ( $P=0.68$ ), 0% ( $P=0.09$ ). The 2-year progression free survival and overall survival were 49.0% and 49.9% for CyBor group and 18.8% and 31.3% for the control group (Figure 1). The 2-year GvHD and disease-free survival (GRFS) were 45.6% and 20%, respectively. Despite the limitations of our study that include its size and its design and the delayed neutrophil and platelet engraftment associated with the CyBor regimen in comparison to calcineurin-based prophylaxis, our data confirm the promising outcomes previously reported with the CyBor combination and reaffirm the need for a large randomized study comparing CyBor to a standard calcineurin-based regimen.

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#### Graphs:

Figure 1: Kaplan Meier Survival Curves.



**Disclosure of conflict of interest:** Amhad Samer Al-Homsi: funding from Millennium Pharmaceuticals.

#### P210

##### Prevalence of clostridium difficile and acute graft-versus-host disease in patients with AML or MDS undergoing allogeneic hematopoietic stem cell transplantation

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Clostridium difficile infection (CDI) causing enterocolitis may represent a serious clinical problem in patients undergoing allogeneic hematopoietic cell transplantation (allo HCT). The reported prevalence varies substantially among heterogeneous patient cohorts. Although CDI has been proposed as a risk factor for the development of gastrointestinal (GI) acute graft-versus-host-disease (aGvHD), limited knowledge on the prevalence of CDI, occurrence of GI aGvHD in CDI patients, relapse incidence and mortality of CDI patients in large patient cohorts is available. The aim of this analysis was to study the implications of CDI in a homogenous cohort of patients with either AML or MDS undergoing allo HCT. At our center all patients undergo stool test once a week for Clostridium difficile antigen while in aplasia until discharge, irrespective of clinical symptoms for enterocolitis. Patients with positive stool antigen tests (that is, toxin test) in the absence of clinical symptoms were referred to as CD+, in contrast to patients without a positive test and without clinical symptoms which were referred to as CD-. We retrospectively analyzed the data of a total of  $n=727$  patients with either AML or MDS undergoing allo HCT in our institution between 2004 and 2015. Overall survival (OS) was measured from allo HCT to the date of death or last follow-up. After HSCT, relapse and non-relapse mortality were considered as competing events. Event-probabilities were calculated according to Kaplan-Meier for OS and using competing event statistics for the cumulative incidence of relapse (CIR), non-relapse mortality (NRM) and aGvHD. 95% confidence intervals (CI) were provided for major endpoints. Statistical analyses were performed using the R environment for statistical computing version 3.1.3 (R Core Team 2015, Vienna, Austria, www.R-project.org). From a total of  $n=727$  patients with either AML or MDS who underwent allo HCT, we identified  $n=528$  (73%) who were CD-,  $n=103$  (14%) who were CD+, and  $n=96$  (13%) who had CDI. Interestingly,  $n=33$  (34%) of patients with CDI were diagnosed having GI aGvHD as compared to  $n=13$  (13%) of patients who were CD+ and compared to  $n=95$  (18%) of patients who were CD-,  $P=0.001$ . The three groups harbored no differences when comparing incidences of liver and skin aGvHD or chronic GvHD, respectively. When dissecting GI aGvHD according to CTCAE criteria, only  $n=8$  (24%) of CDI patients vs  $n=7$  (54%) of CD+ patients, and  $n=53$  (56%) of CD- patients had grade 3–4 GI aGvHD,  $P=0.007$ . With regard to OS and TRM, no statistical differences were observed between the three groups. The CIR was 13% for patients with CDI, 15% for CD+ patients and 9% for CD- patients,  $P=0.02$ , respectively. This analysis represents the largest published analysis of Clostridium Difficile in patients with AML or MDS who underwent allo HCT. The prevalence of CDI in this patient cohort was 13%. Patients with CDI developed significantly more often GI aGvHD as compared to patients who were either CD+ or CD-, respectively. However, this did not translate into differences in OS or TRM.

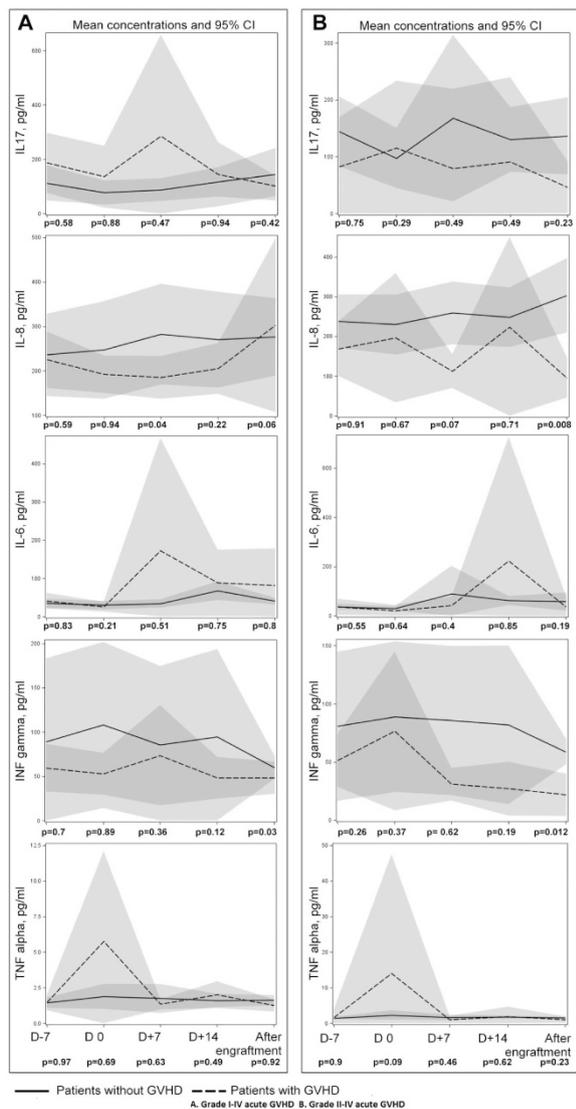
**Disclosure of conflict of interest:** Friedrich Stölzel has received research funding from Astellas.

#### P211

##### Profiles of IL-17, IL-6, IL-8, IFN- $\gamma$ and TNF- $\alpha$ in allogeneic stem cell transplantation with posttransplant cyclophosphamide

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The majority of studies on cytokines in allogeneic HSCT were performed with classical GVHD prophylaxis, consisting of non-specific immunosuppressive agents. With this type of prophylaxis almost in all studies published, higher levels of pro-inflammatory cytokines are associated with development of acute GVHD, while lower levels indicate the success of immunosuppressive agents in abrogation of alloreactive response. Currently, there is no data, whether the dynamics of cytokines after PTCy is similar to the situation of classical GVHD prophylaxis. Out of 192 adult patients transplanted at First State Medical University with PTCy between 2014 and 2015 we have identified 20 cases with acute GVHD and plasma samples available. These patients were matched in the ratio 1:2 to patients who did not develop acute GVHD. The study group was comprised of 60 adult patients with hematological malignancies who underwent HSCT. All patients received PTCy-based GVHD prophylaxis. Five plasma biomarkers were studied by ELISA: IL-17A, IL-6, IL-8, TNF- $\alpha$  and IFN- $\gamma$ . Blood samples were obtained from patients on days -7, 0, +7, +14. The fifth time point varied between day +21 and +28 to represent the sample after engraftment, but before onset of acute GVHD. About 10 (50%) out of 20 GVHD patients had a grade I, 7 (35%) grade II, 5 (25%) grade III aGVHD, 6 (30%) patients developed multiorgan aGVHD. About 13 patients

(21.6%) had chronic GVHD. There was no difference between GVHD + and GVHD - groups in any of the clinical parameters. The median of engraftment for all patients was 21 (9-43: range). The median aGVHD was 30 days (23-92: range). Neither of the cytokine levels was significantly different in patients with aGVHD grades I-IV and without GVHD. However, for patients with aGVHD grade II-IV we found that low levels of IL-8 on day +7 ( $126.83 \pm 43.794$  vs  $276.89 \pm 310.51$  pg/ml,  $P=0.04$ ) and IFN- $\gamma$  on day +21-28 ( $34.70 \pm 23.71$  vs  $60.96 \pm 41.37$  pg/ml,  $P=0.03$ ) were associated with increased risk of GVHD. The ROC analysis was performed to determine the cut off values for IL-8—133.56 pg/ml (AUC = 0.714) and IFN- $\gamma$ —35.94 pg/ml (AUC=0.720). The incidence of aGVHD grade II-IV was significantly higher in patients with levels of cytokines lower than cut off (40% vs 5.7%,  $P=0.008$  and 43.7%,  $P=0.012$  for IL-8 and IFN- $\gamma$ , respectively). The same pattern was observed for patients with aGVHD grade III-IV. Low levels of IL-8 ( $96.12 \pm 39.79$  vs  $303.52 \pm 346.19$  pg/ml,  $P=0.008$ ) and IFN- $\gamma$  ( $21.69 \pm 14.78$  vs  $58.80 \pm 39.92$  pg/ml,  $P=0.012$ ) on day +28 were especially predictive. The cut off values for IL-8 was 147.09 pg/ml (AUC = 0.869) and for IFN- $\gamma$ —25.71 pg/ml (AUC = 0.858). The incidence of aGVHD grade II-IV was also significantly higher in patients with levels of cytokines lower than cut off ( $P=0.004$  and  $P=0.0006$  for IL-8 and IFN- $\gamma$ , respectively). For chronic GVHD only higher level of IL-17 at day +28 ( $209.17 \pm 329.59$  vs  $106.06 \pm 210.65$  pg/ml,  $P=0.037$  for patient with and without GVHD, respectively) was significantly predictive. In this pilot trial we have demonstrated that dynamics of cytokines after GVHD prophylaxis with PTCy may be different from conventional one, and well-known predictive biomarkers might not work after PTCy. Further large prospective trials are warranted to elucidate reliable biomarkers for GVHD after this type of prophylaxis.

**Disclosure of conflict of interest:** None.

**P212 Promising efficacy and safety profile of ruxolitinib in highly pre-treated chronic GvHD patients**

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Graft versus Host Disease (GvHD) remains one of the main obstacles to broader application of allogeneic transplantation. GvHD prevention and treatment techniques are poorly standardized. The 1st-line treatment of newly diagnosed chronic (c) GvHD is corticosteroid. There is no standard 2nd-line treatment for cGvHD. Approximately 50-60% of patients (pts) with cGvHD require secondary treatment within 2 y after initial systemic treatment. Recently the JAK1/2 inhibitor ruxolitinib emerged as an efficacious treatment for corticosteroid-refractory (SR) acute and c-GvHD with a 24% of SR-cGvHD patients reporting a long lasting immunosuppression-free complete response. The current study seeks to analyse the efficacy and safety of ruxolitinib in highly pre-treated SR-cGvHD pts in our Centre. Ruxolitinib treatment was given off label after provision of an informed signed consent and in the absence of alternative therapeutic options including clinical trials. We analysed data prospectively collected at our long-term follow-up clinic between 2015 and 2016. A written consent was given by pts allowing the use of medical records for research in accordance with the Declaration of Helsinki. Overall 5 pts (median age 57 y—range: 39-67 years; mean Karnofsky score 70%) with SR-cGvHD were treated

with ruxolitinib. Median time from transplant was 46 months (range: 9–68). Ruxolitinib was initiated at a starting dose of 5 mg twice daily—median time on ruxolitinib 4 months (range: 2–15)—4/5 pts increased the dose up to 10 mg twice daily. Four pts had a classic and 1 an overlap SR-cGVHD. All of them had skin sclerodermatous involvement and 4/5 joint and fascia involvement with significant decrease of range of motion and limitation of ADL. All pts were previously treated with several lines of immunosuppression (3–11) including high-dose prednisone in 1st line (5/5), rapamycin (5/4), TK-inhibitor imatinib (4/5), extracorporeal photopheresis (4/5). All pts were pre-screened for risk of infection and regularly checked on a fortnightly basis. All pts were under active prophylaxis according to recommendation for GVHD pts and ruxolitinib therapy. After a cumulative follow-up of 867 days we reported only one serious adverse event represented by a CMV pneumonia requiring hospitalization with complete recovery. Early time point evaluation (5/5 pts evaluable) at +1 month underlined how all pts were reporting subjective improvement at the patient global ratings according to NIH 2014. Data were confirmed at the health care provider global ratings. Month 3 evaluation (3/5) confirmed meaningful responses (partial responses 3/3) according to NIH 2014, with both patient and health care provider global ratings improvement and concomitant enhancement in Lee skin symptoms score and SF-36 health-related QoL. At last follow-up no evidence of myelosuppression, infections, PML, non-melanoma skin cancer was registered. Considering the concomitant treatment (with reference to azoles and rapamycin or cyclosporine) no cases of toxicity due to drug-drug-interaction was reported. Ruxolitinib is well tolerated in highly pre-treated SR-cGVHD. Its safety profile seems to be reassuring. The efficacy data observed also at this early time point is preliminary but promising in this subset of pts with a long history ( $\geq 3$  lines) of treatment for cGVHD. Confirmatory study in a larger number of patients is underway on a multicentre basis.

**Disclosure of conflict of interest:** None.

#### P213

##### Previously published

#### P214

##### Ruxolitinib in children with steroid-refractory severe acute enteral GVHD

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Severe acute enteral graft-versus-host-disease (GVHD) is a life-threatening complication of allogeneic bone marrow transplantation. In case of resistance to corticosteroids as the first-line treatment severe enteral GVHD harbors a high morbidity and mortality. Retrospective analyses indicate efficacy of the JAK1/2-inhibitor ruxolitinib in the treatment of acute or chronic GVHD in adults, but experience in paediatric patients is limited. Here, we report a small cohort of paediatric patients with stage 4 steroid-refractory GVHD of the gut who received ruxolitinib as salvage therapy within a multimodal immunosuppressive regimen. We retrospectively analysed four patients aged 8–16 years with severe, steroid-resistant acute GVHD of the gut who were treated with ruxolitinib in our institution. All patients were transplanted for non-malignant haematologic disorders, graft source was 2 × MMUD, 1 × MUD, 1 × MSD. The conditioning regimen consisted of treosulfan, fludarabine and thiotepa. Serotherapy with thymoglobuline was administered in all patients transplanted from unrelated donors. All patients received MTX and cyclosporine as GVHD-prophylaxis. GVHD was staged according to the Glucksberg-Scale. Ruxolitinib was added to the immunosuppressive regimen when acute stage 4 GVHD was reached and became resistant to treatment with methylprednisolone (2 mg/kg/day)

as well as infliximab and mycophenolate (MMF) as second-line immunosuppressants. Acute stage 4 enteral GVHD developed at a median of 38 days after transplant (30–58 days) and ruxolitinib was started at a median of 53 days post-transplant (48–85 days). The starting dose varied between 10 mg/day and 40 mg/day, that is, 0.25–0.5 mg/kg/day, taking into account the expectedly low bioavailability of the oral drug during severe diarrhea. Upon improvement of GVHD symptoms and/or increasing side effects the dose was gradually tapered and ruxolitinib was discontinued after a median of 39 treatment-days (19–83 days). After addition of ruxolitinib to the immunosuppressive regimen, the symptoms of acute gut GVHD gradually improved in all four patients with decreasing abdominal pain and stool volumes. Immunosuppression with steroids and MMF could slowly be tapered. All patients are alive after a median follow-up of 392 days (95–571 days) from diagnosis of acute stage 4 gut GVHD. The most prominent side effect attributable to ruxolitinib was thrombocytopenia with a nadir in platelet counts after 30 days of ruxolitinib treatment in 3/4 patients. Platelets recovered within 2 weeks after ruxolitinib was discontinued. Neutropenia was observed in one patient with ANC dropping  $< 0.5/\text{nl}$  after 30 days of ruxolitinib treatment. Mild to moderate elevation of liver transaminases was observed in all four patients during ruxolitinib treatment. One patient developed imminent acute renal failure, another patient showed symptoms of hemolytic uraemic syndrome. However, due to the multimodal treatment of these critically ill patients, these complications could not clearly be attributed to ruxolitinib. Ruxolitinib is potentially beneficial in severe acute enteral GVHD in children refractory to corticosteroids as well as second-line immunosuppressants. However, randomized trials are warranted to verify safety and efficacy of ruxolitinib in this patient cohort.

**Disclosure of conflict of interest:** None.

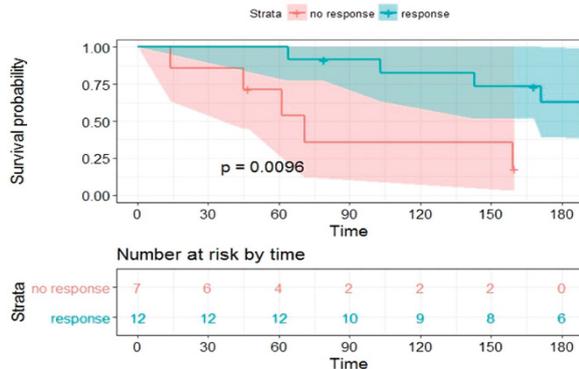
#### P215

##### Ruxolitinib plus extracorporeal photopheresis (ECP) may increase response in steroid refractory acute graft-versus-host disease (aGVHD)

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Steroid refractory acute GVHD is a major cause of mortality after allogeneic stem cell transplantation. Until date, no agent or treatment strategy has demonstrated superior efficacy in this patient group. The dose and duration of steroid treatment is associated with several short and long-term side effects, therefore concepts facilitating rapid steroid taper may be beneficial. Both Ruxolitinib and ECP have been reported to be effective in treatment of steroid refractory (SR) aGVHD. We analyzed data from consecutive adult patients who received Ruxolitinib for SR aGVHD between March 2015 and August 2016 in our institution. Overall, 19 patients (male  $n = 12$ ; female  $n = 7$ ) with a median age of 58 years (range: 18–74) were included. Donors for allogeneic SCT were MSD ( $n = 3$ ), MUD ( $n = 12$ ) and MMUD ( $n = 3$ ). Median time to GVHD onset after stem cell transplantation was 29 days (range: 7–154 days). About 14 patients had aGVHD grade III or IV (all with GI involvement), while 5 patients had skin grade 3 involvement. SR aGVHD was diagnosed if aGVHD manifestations were progressive after 3 days or persistent and without improvement after 7 days or no partial remission after 14 days of treatment with 2 mg/kg BW of systemic steroids. Patients received additional ECP ( $n = 11$ ), if response to Ruxolitinib was lacking or slow ( $n = 9$ ) or instead of Ruxolitinib due to cytopenias ( $n = 2$ ). Ruxolitinib was first-line treatment for SRaGVHD in 11 patients (58%). Median initial dose of ruxolitinib was 10 mg (range: 5–15 mg) twice daily. Steroids were tapered and stopped, even if aGVHD was still active. Primary end point was non-relapse mortality at 6 months. Secondary end point was response on day 28 after initiation of Ruxolitinib. Response occurred relatively slowly, resulting in a

day 28 overall response rate of 58% (CR=6, PR=5). However, a total nine patients (47%) attained a complete response (CR), five with ruxolitinib alone and four others in combination with ECP. About 12 patients (63%) required dose reduction or interruption of ruxolitinib mainly due to cytopenias. After a median follow-up of 210 days, 8 patients are alive. Causes of death were relapse of malignant disease ( $n=1$ ), GVHD ( $n=2$ ), infections ( $n=7$ ) and other ( $n=1$ ). Median survival from diagnosis of SR aGVHD was 61 days for non-responders and 252 days for responders (Figure 1,  $P=0.0096$ ). In univariate analysis, non-response was associated with higher risk of non-relapse mortality (RR; 5.6, 95% CI: 1.51–20.6,  $P=0.01$ ). Ruxolitinib and ECP are two effective promising treatment options, which may be complementary in patients with SR aGVHD. Cytopenia is the most frequent side effect of ruxolitinib while infections remain the major cause of death. [P215]



**Disclosure of conflict of interest:** Ayuk—Therakos: Honoraria; Kröger: Novartis: Honoraria, research funding.

**P216**  
**Ruxolitinib treatment for corticosteroid-resistant acute intestinal GVHD**

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Steroid-refractory acute graft-versus-host disease (SR-aGVHD) is associated with a dismal outcome. Janus kinase (JAK) 1/2 signaling has been shown to be instrumental in multiple steps leading to inflammation and tissue damage in GVHD. JAK1/2 inhibitor ruxolitinib was studied in the treatment of SR-GVHD by Zeiser *et al.* (*Leukemia* 2015), and the overall response rate was reported to be 81.5%. We have now studied ruxolitinib in the treatment of six adult patients with steroid-refractory, grade III–IV, intestinal aGVHD. All the patients were male. The median age of the patients was 50 (range: 19–59) years. Three of the patients were transplanted for AML, one for ALL, MDS and MM each. All the patients had been given a myeloablative conditioning treatment (CyTBI 2, Treosulfan+Fludarabine 4). Two patients had a sibling donor and four a matched unrelated donor. The graft was from peripheral blood in all the patients. GVHD prophylaxis consisted of cyclosporine and a short course of methotrexate, and in addition antithymocyte globulin in the unrelated donor setting and methylprednisolone in one sibling recipient. aGVHD of the intestine manifested on days +17, +25, +39, +60, +63 and +136 with diarrhea. In two patients it was preceded by aGVHD of the skin by 7 and 49 days, respectively. GI-biopsy showed acute GVHD of grade III and of grade IV in three patients each. Treatment of intestinal GVHD was started with methylprednisolone 10 mg/kg/day, tapering the dose to 5 and 2 mg/kg after 12 doses each. Gastroduodenoscopy and colonoscopy were performed at the onset of symptoms indicating intestinal GVHD. Biopsy

confirmed the diagnosis in all cases. Because the diarrhea continued in spite of methylprednisolone treatment, ruxolitinib was started 7, 9, 10, 10, 20 and 90 days from the first day of diarrhea. The dose of ruxolitinib was 10 mg × 2 per day orally. Four patients showed a clear response to ruxolitinib, normalization of bowel function, after 3, 4, 16 and 27 days from the start of ruxolitinib treatment. The healing of the intestinal lesions was verified by biopsy. Two of these patients had received extracorporeal photopheresis simultaneously. Two patients did not benefit from ruxolitinib treatment. One of them had continuous infectious complications and therefore ruxolitinib was only started after 90 days from the start of diarrhea. The other patient died of fulminant diarrhea after 3 weeks of ruxolitinib treatment. CMV reactivation was detected in three of the responders, and two of them had also polyoma virus cystitis. One patient developed a pulmonary aspergilloma, which is under control with drugs. Corticosteroid-resistant gastrointestinal acute GVHD was treated in six patients, out of whom four showed a good response.

**Disclosure of conflict of interest:** None.

**P217**  
**Safety and efficacy of low-dose methotrexate for the treatment of refractory sclerodermatous chronic graft-versus-host disease: a single institution retrospective study**

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Although methotrexate (MTX) is commonly used in the prophylaxis of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT), some small studies have also reported its use in the treatment of chronic GVHD. The aim of this study was to evaluate the efficacy and safety profile of low-dose MTX for treatment of sclerodermatous chronic GVHD (sGVHD) after the failure of first and second line treatments. We retrospectively evaluated 23 adult patients who received low-dose MTX as salvage treatment of sGVHD during the period elapsed between June 2006 and June 2016 in a Tertiary Referral University Hospital in Spain. There were 17 (73%) males and 6 (27%) females. The median age was 54 years (range: 28–69). All had received an allo-HSCT for hematologic malignancies. The median time from allo-HSCT to sGVHD was 666 days (range: 334–2679). Thirteen patients (56%) had presented previous acute skin GVHD. Superficial skin lesions mimicking lichen planus (lichenoid GVHD) were diagnosed in 19 (82%) patients, while lesions resembling lichen sclerosis, morphea or fasciitis (sGVHD) were seen in all 23 (100%) patients. The total body surface area was affected by more than 50% in 15 patients (65%). Besides the skin, other organs/tissues involved were the eyes (65%), mouth (52%), nails (34%), lungs (17%), liver (8%) and gastrointestinal tract (4%). Treatment lines prior to MTX administration were: prednisone (PDN) in 23 patients (100%), phototherapy (PhT) in 4 (17%), cyclosporine (CyA) in 2 (8%), mycophenolate mofetil (MMF) in 2 (8%), PhT + PDN + CyA in 3 (13%), PDN + MMF + CyA in 3 (13%), extracorporeal photopheresis + PDN in 1 (4%). The median time from sGVHD onset to MTX treatment was 308 days (range: 19–937 days). MTX was administered subcutaneously in 21 patients (91%) and orally in 2 patients (9%). Median dose of MTX was 13.74 mg/week (range: 7.73–18.48 mg/week) and median length of treatment was 61 weeks (range: 2–148 weeks). In two patients (8%) early withdrawal of MTX occurred (one due to early death secondary to septic shock and other due to rapid disease progression). MTX-related toxicity occurred in three patients (13%): megaloblastic anemia, asymptomatic increase of liver enzymes and mucositis, respectively. Response to MTX was evaluated in the 21 patients (91%)

who did not suffer early MTX discontinuation. Seventeen patients (73%) presented a partial response; of them, two are still under MTX treatment for 26 and 59 weeks, respectively. Fourteen patients (60%) received PDN concomitantly to MTX (median dose 20 mg/day, range: 5–60); 1 year after MTX treatment, only four patients were receiving PDN (median dose 5 mg/day, range 5–15). Seven patients have finished MTX treatment without reappearance of symptoms, receiving only topical treatment with emollients, tacrolimus or corticoids for short periods. In four patients (26%) sGVHD progressed despite MTX administration. Our data suggest that MTX is a safe, inexpensive and effective alternative for refractory sGVHD. Its potential used in earlier phases of sGVHD deserves further investigation.

**Disclosure of conflict of interest:** None.

#### P218

##### **Successful outcome after extracorporeal photochemotherapy (ECP) in persistent refractory chronic graft versus host disease (cGVHD)**

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Severe chronic GVHD has a major influence on late morbidity and mortality after hematopoietic stem cells transplantation (HSCT). ECP is a good approach to treat refractory-GVHD: leucocytes are obtained from peripheral blood by apheresis, incubated with 8-MOP, irradiated and then infused to the patient where they undergo apoptosis and induce tolerance. It is a promising alternative that reduces doses of immunosuppressive therapy and their side effects in the treatment of GVHD. This study shows its efficacy in persistent refractory cGVHD. The procedure was applied to three patients (pts) aged 24, 28 and 32 years (two AML and one CML), sex ratio (1M/2F) who underwent allogeneic-HSCT with myeloablative conditioning regimen based on chemotherapy alone from a peripheral blood stem cells with CD34 levels: 1.24, 7.6 and  $8.23 \times 10^6/\text{kg}$  respectively. Prophylaxis of GVHD combined ciclosporin and methotrexate in short cycle. Severe extensive cGVHD (according to NIH criteria) was observed in the three cases after an average delay of 5.3 months (3–7) with involvement of 1–6 organs (mouth, eyes, skin, liver, joints and lungs). All pts are refractory to three lines of immunosuppressive agents (ciclosporin–corticosteroids, MMF and Imatinib), with an average of 3 thrusts/pt (2–4) over an average period of 65 months (09–103). ECP was performed under the open system or dissociated system (Macopharma) for two sessions per week for 4 weeks, one session per week for 8 weeks, one session every 2 weeks for 12 weeks and one session per month for 3 months. After a median period of 6 months (3–10), an average of 22 sessions/pt (15–26) was performed. In terms of tolerance, a red blood cell transfusion was required in one pt, spontaneously resolved lymphopenia was observed in another pt, and a poor venous approach led to the pause of a central catheterization in one pt. The 3-month and 6-month evaluation according to the Couriel response criteria shows a partial response observed as of the first month with net improvement especially on skin sclerotic features and joints retractions initially refractory to all therapeutic lines. This allowed gradual reduction doses of corticosteroids. PCE is recommended in the curative treatment for refractory chronic GVHD from the second line. This encouraging study on a small series shows its efficacy in persistent and late refractory forms. It is nevertheless necessary to evaluate it on a larger number of pts.

**Disclosure of conflict of interest:** None.

#### P219

##### **Successful treatment of steroid-refractory acute gastrointestinal graft-versus-host-disease by fecal microbiota transplantation**

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Steroid-refractory acute gastrointestinal (GI) graft-versus-host disease (aGVHD) is a severe complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) associated with a high mortality rate. Loss of intestinal bacterial diversity is thought to be associated with severity of GI-aGVHD and an impaired intestinal microbiota with reduced diversity is an independent predictive factor for mortality. Fecal microbiota transplantation (FMT) is the application of a fecal suspension derived from a healthy donor into a patient's GI tract. It has been successfully applied in recurrent *Clostridium difficile* associated diarrhea including patients who underwent allo-HSCT. We report the complete resolution of lower GI-aGVHD following colonoscopic FMTs in three patients that had been refractory to 4–6 lines of immunosuppressive therapies. Microbiota analysis by 16s rDNA before FMTs revealed a severely depleted microbiota in all patients. Donors (different persons for each patient) were healthy adult subjects. Repetitive (1–6) colonoscopic FMTs were necessary to permanently establish the donor's microbiome. All patients responded clinically by reduction/normalisation of stool volumes, stopping total parenteral nutrition and tapering of steroids. A possible causative relationship of FMT in the reversal of severe intestinal dysbiosis and subsequent resolution of GI-aGVHD can therefore be hypothesized. The establishment of donors' microbiota and increase in bacterial richness was associated with disease control. No immediate procedure-related infections or other side effects were observed. Besides restoration of an initially severely reduced microbial richness by FMTs, response of GI-aGVHD was sustained despite reduction and discontinuation of concomitant immunosuppressive treatments. Restoration of dysbiosis by FMT might represent a promising novel therapeutic approach for refractory lower GI-aGVHD.

**Disclosure of conflict of interest:** None.

#### P220

##### **Tear film proteomics reveals important differences between patients with chronic ocular GvHD and healthy controls**

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Chronic GvHD frequently involves the eyes, leads to important decrease of quality of life and may threaten visual capacity. Sicca syndrome is one of the hallmark of ocular cGVHD. Analysis of tear protein composition may help to identify biomarkers for early diagnosis and prognosis of ocular cGVHD. Tear fluid of 42 patients with ocular cGVHD were compared with 10 healthy individuals in this single center study. Results of the first 10 patients are reported here. Tryptic digests from Schirmer strips were analyzed on an Orbitrap mass analyzer. Clinical examinations included slit lamp examination, fluorescein staining, Schirmer test, break-up-time (BUT) and a quality of life questionnaire (OSDI). Outcome measures were differences and consistency of proteins in human tear fluid

between patients with ocular GvHD and healthy controls. Statistical analysis was performed by one sample Wilcoxon-Tests,  $P$ -values  $< 0.01$  was considered significant. Ten patients (eight males, two females) with a median age of 47 years (range: 24–69) were analyzed. All underwent PBSCT, eight from an unrelated donor. cGvHD overall score was moderate in three and severe in seven. Eye organ score was 2 in six and 3 in four patients. All patients had more than one organ manifestation of cGvHD. Eight were under systemic immunosuppressive therapy at the time of analysis, two had topical treatments only. In total 306 different proteins were detected in tears analyzed. Compared to controls, 172 were differentially expressed in ocular cGvHD. Expression was highly significantly different in 75 proteins. Compared to controls, expression of 41 proteins was at least 10-fold increased, representing 11 different categories. Among them, more than 75% of all proteins belong to one of three categories: cytoskeletal proteins, nucleic acid binding or structural proteins. Albumin, cluster or keratin (keratin type I–III) and cluster of pyruvate kinase were most highly overexpressed. Expression of 14 proteins was decreased to  $< 1$  to 50%, belonging to 12 different protein classes. Half of them belong to defense/immunity proteins, enzyme modulators, hydrolases, nucleic acid binding and carrier proteins. Expression of lactotransferrin, proline-rich protein and prolactin-inducible protein was most profoundly decreased. Compared to healthy controls, a high number of protein is found to be differentially expressed in tears in ocular cGvHD. Among them high expressions are observed for proteins that may indicate disturbed integrity of ocular surface and leakage of conjunctival capillaries. Most profoundly decreased proteins include proteins with important functions in host defense and immunomodulation. More detailed pathway analysis is necessary to identify biomarkers for ocular cGvHD.

**Disclosure of conflict of interest:** None.

#### P221

##### **The restoration of naive recipient immune system in steroid-dependent cGvHD treatment: a clinical case**

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Steroid-dependent chronic GvHD after allogeneic peripheral blood stem cell transplantation is a great problem. Non-responders to corticosteroid therapy are at high risk of mortality. We hypothesized that such patients could benefit from treatment strategy using in patients with primary severe autoimmune diseases like multiple sclerosis and Crohn's disease. Patient Z., 4 y.o. was diagnosed in July 2011 with myelomonocytic leukemia (JMML). Initially he was treated with low-dose of cytarabine and epigenetic agents. In September 2013, JMML progression was observed with leukocytosis, thrombocytopenia, splenomegaly. Bone marrow aspirate showed 19.2% monocytes and 19.8% blast cells. Splenectomy was performed in November 2013 due to refractoriness to blood components transfusions. In December 2014 unmanipulated haploidentical peripheral blood stem cell transplantation from mother with  $3.2 \times 10^6/\text{kg}$  CD34+ and  $3.5 \times 10^8/\text{kg}$  CD3+ was performed. The conditioning regimen was myeloablative including melphalan  $100 \text{ mg}/\text{m}^2$  day - 5 and treosulfan  $14\,000 \text{ mg}/\text{m}^2$  days - 4, - 3, - 2. No organ toxicity  $>$  grade 2 was observed. GvHD prophylaxis consisted of hATG  $10 \text{ mg}/\text{kg}$  on days - 5, - 3, - 1, + 1, i.v. tacrolimus from d - 1 and MMF  $25 \text{ mg}/\text{kg}$  from d 9. Engrafted was fast and prompt (100% donor) with  $\text{WBC} > 1.0 \times 10^9/\text{L}$  on d + 8,  $\text{PLTs} > 20 \times 10^9/\text{L}$  on d + 10. Acute GvHD of stage II was observed in early posttransplant period and treated with steroids and TNF- $\alpha$  inhibitor (infliximab). Patient also received five procedures of ECP. All attempts of immunosuppression tapering failed and the patient was staying on high dose of tacrolimus, MMF and courses of steroids till October 2015. In October 2015, GVHD

stage II flare with blood eosinophilia occurred after another attempt of steroids withdrawal. Clinical examination showed that the patient was in complete remission with full donor chimerism. Mild response of GVHD to steroids was observed. In April and May 2016 patient received two doses of rituximab  $375 \text{ mg}/\text{m}^2$  with no significant response. In order to restore naive immune system first course of chemotherapy with cyclophosphamide  $2000 \text{ mg}/\text{m}^2$  was performed in the end of May 2016. No toxicity  $>$  grade 2 was observed. The patient recovered  $\text{WBC} > 1.0 \times 10^9/\text{L}$  on d + 12,  $\text{PLTs} > 20 \times 10^9/\text{L}$  on d + 11. In the phase of hematological recovery he was mobilized with G-CSF and two leukaphereses of PBSCs were performed. In June 2016 our patient was transplanted with previously collected  $0.5 \times 10^6$  CD 34+/kg following nonmyeloablative regimen including cyclophosphamide  $1500 \text{ mg}/\text{m}^2$  on day - 3 and fludarabine  $10 \text{ mg}/\text{kg}$ , on days - 3, - 2, - 1. Second dose of cyclophosphamide was not administered because of severe hyponatremia with seizures due to the CPM administration. No other significant toxicity was observed. The patient did not require either blood product or i.v. antibiotics. Doses of tacrolimus and MMF were picked on 2 months late and no more steroids were given. The patient is well in CR with no signs of GvHD for 7 months. We speculate that PBSC collection from patients under massive immunosuppression underwent allogeneic transplant is difficult but feasible. The nonmyeloablative regimens in such group of patients could be well tolerated and ensure the restoration of naive recipient immune system. This option could be discussed as an attractive alternative for treating resistant GvHD in steroid resistant patients.

**Disclosure of conflict of interest:** None.

#### P222

##### **The role of 18F-FDG PET/CT as a non-invasive modality in the diagnosis of acute gastrointestinal graft versus host disease (GI-GvHD) in children**

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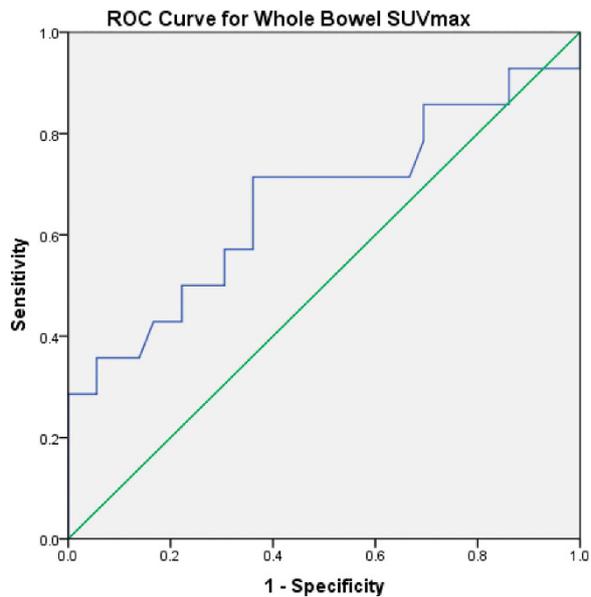
Severe acute GI-GvHD is a serious early complication of allo-transplants, and still remains a clinical diagnosis. (1) Endoscopic biopsies provide the best supportive evidence, but are invasive and morbid in patients who are already medically compromised. <sup>18</sup>F-FDG PET/CT may be able to stratify patients who require endoscopy and biopsy. To evaluate the performance of <sup>18</sup>F-FDG PET/CT in differentiating moderate to severe GI-GvHD from no or mild disease in pediatric patients with suspected GI-GvHD. Retrospective chart review of all paediatric allo-transplant patients referred for <sup>18</sup>F-FDG PET/CT with suspected GI-GvHD from 2009 to 2015. Clinical follow-up, endoscopy and biopsy findings were correlated with <sup>18</sup>F-FDG PET/CT. Regional SUV parameters were extracted by placing ROIs around stomach, duodenum, distal ileum, caecum, ascending, transverse, descending colon, recto-sigmoid colon and rectum. Regional, and average large and small bowel SUV data were statistically compared between patients with no or mild GIT-GvHD vs moderate to severe disease. The clinical and biopsy-supported diagnosis of acute GI-GVHD was taken as the true positive diagnosis for acute GI-GVHD. ROC curves were generated for whole bowel SUVmax values. About 50 scans in 34 patients, median age of 9 years (6 mths to 18 y), were performed at a median of 71 days post BMT. There were 15 stage 1, 13 stage 2–4 and 22 with no acute GI-GvHD. Transverse colon SUVmax was significantly higher in the stage 2–4 GI-GvHD compared to no or Stage 1 disease (Mann-Whitney-U-test  $P < 0.05$ ). There was a non-significant trend for average large bowel SUVmax to be higher in the Stage 2–4 group than the no or Stage 1 disease group (mean SUVmax 4.16 compared to 2.94,  $P = 0.07$ ). A cut off whole bowel

SUVmax 2.74 had a sensitivity of 79% and specificity of 61% for detecting moderate to severe GI-GvHD. <sup>18</sup>F-FDG PET/CT is a feasible and potentially useful non-invasive tool in the diagnosis and monitoring of therapeutic efficacy in acute GI-GvHD (2). Large bowel SUVmax may be higher in patients with stage 2–4 GI-GvHD, and transverse colon SUVmax could have the ability to differentiate children with no or stage 1 GI-GvHD from those with stage 2–4 disease. A negative <sup>18</sup>F-FDG-PET/CT could serve as a criteria to avoid invasive endoscopic procedures and observe for the persistence of gastrointestinal symptoms before subjecting these patients to an image-guided biopsy. In patients too unwell for endoscopy, SUVmax >4 (ROC curve Specificity 75%) and a high SUVmax in the transverse colon could serve as supportive evidence for moderate to severe acute GvHD, in the absence of biopsy findings. A major advantage of a pre-endoscopic <sup>18</sup>F-FDG PET/CT is to guide the proceduralists to sample areas with the best diagnostic yield. Prospective controlled studies are needed.

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[P222]



Diagonal segments are produced by ties.

**Disclosure of conflict of interest:** None.

#### P223

##### The role of stromal dysfunction in oral chronic GVHD

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Oral mucosal progenitor cells (OMLP-PCs) possess immunomodulatory and antibacterial properties, suggestive of their *in vivo* function in healthy tissue and their potential contribution to scarless wound healing in the buccal mucosa (2, 3). Our aim was to establish whether the function of oral stromal progenitors is impaired in chronic graft versus host disease (cGVHD) and restored with response to treatment. A patient with grade 3 oral cGVHD was treated with systemic thalidomide for 9 weeks (200 mg/day). Punch biopsies of buccal mucosa were taken before and after treatment. Oral progenitor cells were isolated and expanded *in vitro*. Numbers of progenitors was assessed using colony forming unit-fibroblast (CFU-F) assays. Stem cell markers (CD90, CD105, CD73, CD29, CD34, CD45, HLA I and II) were evaluated by flow cytometry. Wound healing and antibacterial potential were assessed using a collagen gel lattice assay and bacterial co-cultures as previously described (2, 4). Secreted levels of relevant cyto- and chemokines associated with wound healing were assessed by ELISA. Significant clinical improvement with reduced inflammation in the oral mucosa and healing of ulcers was seen after 3 weeks of thalidomide treatment, with continued improvement after 9 weeks. Cell surface expression of CD90 and CD105 on OMLP-PCs was elevated post-thalidomide; markers correlated with stemness and angiogenesis in mesenchymal stromal cells. This correlated with a restoration of wound healing potential and antibacterial function after thalidomide treatment (Figure 1). Figure 1: antibacterial testing demonstrated a loss of antibacterial function against (a) Gram positive and (b) Gram negative micro-organisms in the cGVHD OMLP-PCs that could be completely or partially restored to levels comparable with healthy controls after thalidomide treatment. \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . We demonstrate, for the first time a correlation between clinical improvement of oral cGVHD with thalidomide treatment and restoration of endogenous progenitor cell function. This study highlights the importance of a dysfunctional oral mucosal stroma in the pathogenesis of cGVHD. Further studies should focus on the role of the stroma in promoting cGVHD and the precise mechanisms by which thalidomide is able to restore its functions.

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**Disclosure of conflict of interest:** LD is an inventor of the disclosed OMLP-PC patents, owned by University College Cardiff Consultants Ltd.

#### P224

##### Thymic stromal lymphopoietin levels after hematopoietic stem cell transplantation predicts chronic graft-versus-host disease

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Chronic graft-versus-host disease (cGVHD) is a major cause of late morbidity and treatment-related mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). cGVHD is driven by a Th2 biased T-cell mediated alloreactive immune response that leads to chronic inflammation and fibrosis in various organs. Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived cytokine that mainly affects myeloid cells. Upon stimulation with TSLP, dendritic cells are polarized towards a DC2 phenotype driving Th2 biased immune response. We hypothesized that TSLP is involved in the pathogenesis of cGVHD and that elevated levels of TSLP post-transplant may lead to an increased risk of cGVHD. In the present study, we measured plasma TSLP levels during HSCT to study associations with clinical outcomes including cGVHD. About 38 adult patients undergoing myeloablative HSCT at Rigshospitalet, Denmark, from 2011 to 2013 were included. Diagnoses included AML ( $n=15$ ), ALL ( $n=11$ ), myelodysplastic syndrome ( $n=6$ ), other malignancies ( $n=4$ ) and anemias ( $n=2$ ). Donors were either HLA matching siblings ( $n=11$ ) or MUD ( $n=27$ ). Grafts were either BMSC ( $n=24$ ) or PBSC ( $n=14$ ). Conditioning included TBI ( $n=31$ ) or high-dose chemotherapy alone ( $n=7$ ). Plasma TSLP was measured by ELISA (Abcam) before transplantation, at the day of transplantation and at day +7, +14, +21 and +90 post-HSCT. Monocytes were counted daily, and T, B and NK cells were measured at day +30 and +90 using flow cytometry. About 35 (92%) patients engrafted; acute GVHD grade 2–4 was seen in 20 (53%) patients, and 16 (42%) patients developed severe cGVHD. OAS was 57.9%, TRM 26.3% and relapse rate 15.8%. Median plasma TSLP levels increased from before conditioning (101 pg/ml) to reach a peak at day +21 (313 pg/ml,  $P=0.03$ ), followed by a gradual decline. The plasma levels of TSLP at day +21 were positively correlated with same-day monocyte counts ( $\rho=0.58$ ,  $P=0.006$ ). Approximately half of the patients ( $n=14$ ) experienced an overall rise in TSLP from baseline (100 pg/ml) to day +90 (328 pg/ml). This increase in TSLP was not significantly associated with any transplant-related baseline characteristics. However, patients, who had an increase in TSLP levels from baseline to day +90, had a significantly higher risk of extensive cGVHD compared to those in whom TSLP levels at day +90 were similar or below baseline levels (cumulative incidence of cGVHD: 77% (increased TSLP at day +90) vs 38% (normal/low TSLP at day +90),  $P=0.01$ ). Development of cGVHD was also associated with the nucleated cell dose infused ( $P=0.04$ ) and transplant using PBSC ( $P=0.08$ ). TSLP plasma levels were not associated with acute GVHD, OAS, TRM, relapse rate or numbers of T cell, B cell or NK cells post-transplant. We have found that increased levels of TSLP from baseline to day +90 were associated with an increased risk of extensive cGVHD. This association may be due to the ability of TSLP to polarize the immune system toward a Th2 response. Importantly, the increase in plasma TSLP levels was not associated with any transplant-related characteristics suggesting that TSLP may be an independent predictor of cGVHD. These findings indicate that anti-TSLP treatment may be a new approach to fight severe cGVHD.

**Disclosure of conflict of interest:** None.

## P225

### Thymopoiesis following HCT: a retrospective review comparing interventions for aGVHD in a paediatric cohort

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Acute graft-versus-host disease (aGVHD) is a life threatening complication of allogeneic haematopoietic cell transplantation (HCT), treated with topical and/or systemic corticosteroids. In steroid-refractory aGVHD extracorporeal photopheresis (ECP) can be effective. ECP exposes apheresed mononuclear cells to 8-methoxypsoralen and ultra-violet radiation. Systemic corticosteroids and aGVHD are damaging to thymic tissue. Delayed immune reconstitution, especially of the T lymphocyte compartment, is associated with increased morbidity and mortality.<sup>1</sup> Therefore, management strategies must be effective in treating aGVHD but endeavour to minimise resulting thymic damage. We compare the effect of topical steroid therapy, corticosteroids and ECP on thymic reconstitution following HCT in paediatric patients. Statistical analysis was performed using the Kruskal–Wallis test. About 155 paediatric allogeneic HCTs were performed between June 2010 and April 2016, at the Great North Children's Hospital, Newcastle for malignant and non-malignant disease. We reviewed computerised records to categorise patients into four groups: no aGVHD, mild aGVHD treated with topical steroid, aGVHD treated with systemic steroid, aGVHD treated with ECP. Laboratory data were reviewed to provide values of naive (CD4+ and CD4–)CD45RA+CD27+ T-lymphocytes at 3, 6, 9 and 12 months post-HCT. Values for thymic output for the ECP group were additionally recorded at 3, 6, 9 and 12 months during ECP. Excluded were patients with no available data, those with < 12 months follow-up, those with chronic GVHD, recipient of > 1 HCT or received DLI post-HCT. About 104 patients were included, 42 (40.4%) had no aGVHD, 49 (47.1%) had aGVHD treated topically or systemically, 13 (12.5%) had aGVHD and received ECP. For analysis, the group treated with steroids were divided into those treated with topical therapy and those given systemic steroids. The median values of all groups at each time point (3, 6, 9 and 12 months) are shown (Figure 1). There was a significant difference between the rate of thymopoiesis (measured by the addition of CD4+ and CD4– CD25+CD27+ cells) between all groups (no aGVHD, aGVHD treated with topical or systemic steroids, and aGVHD treated with ECP) at 3, 6, 9 and 12 months post-transplant ( $P=0.002$ ,  $P<0.001$ ,  $P<0.001$ ,  $P=0.001$  respectively). Further analysis excluded those treated with ECP (so including the no GVHD ( $n=42$ ), topical treatment ( $n=23$ ) and systemic steroid treatment group ( $n=26$ )). At each time point  $P=0.001$ ,  $P=0.019$ ,  $P=0.021$  and  $P=0.019$ , respectively, demonstrating a statistically significant difference in time to thymopoiesis between those that had developed aGVHD and those that had not.

Figure 1: Median values for naive T cells (both CD4+CD45+CD27+ plus CD4–CD45+CD27+) at 3, 6, 9 and 12 months shown for all treatment groups. Values for the ECP cohort post ECP therapy also shown.

**Disclosure of conflict of interest:** None.

## P226

### Treatment of steroid-refractory AGVHD (SR-AGVHD): results in a single center

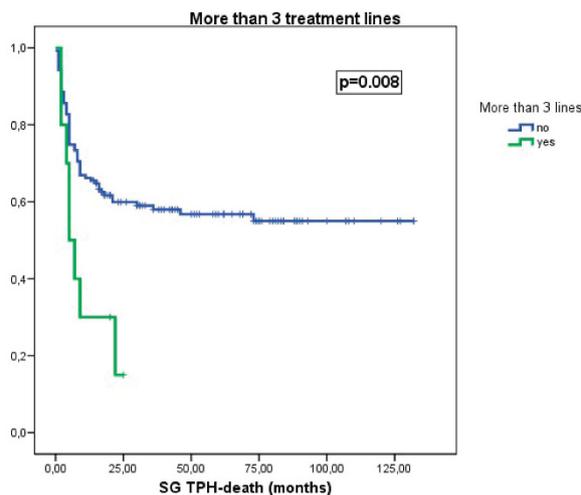
M Yebenes-Ramirez<sup>1</sup>, AI Alvarez-Sanchez, E Garcia-Torres, C Martin-Calvo, FJ Casañó-Sanchez, MA Alvarez-Rivas, R Rojas-Contreras and C Herrera-Arroyo

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Acute graft-versus-host disease (aGVHD) is a significant cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation. Which despite first line treatment is well-established (steroids), second line is not well defined. Evaluate the results with different second line treatment used and the risk factors associated with of SR-aGVHD. We review the clinical records of 281 consecutive patients undergoing allogeneic HSCT from 2005 to 2015 in our hospital. About 53% presented aGVHD. SR-aGVHD was defined as progression after 3 days, no clinical change in 5 days or incomplete response after 14 days of treatment. About 34 patients (25%) met

criteria for SR-aGVHD. There were no significant differences between both groups (SR-aGVHD vs No SR-aGVHD) respect to age (recipient/donor), unrelated donor, prophylaxis of GVHD, CD3 lymphocyte and CD34 cell. The median time between transplantation and aGVHD diagnosis was 25 days (7–123). Patients who did not respond on fifth day of steroid treatment have an 80% rate mortality vs 33% on No SR-aGVHD group ( $P=0.03$ ). SR-aGVHD: 34 patients presented SR-aGVHD and this was related to: HLA mismatch (35% SR-aGVHD vs 15% No SR-aGVHD,  $P=0.008$ ), Male recipient/female donor (38% SR-aGVHD vs 17% No SR-aGVHD,  $P=0.02$ ) and advanced underlying disease (56% SR-aGVHD vs 22% No SR-aGVHD,  $P=0.001$ ). Second line: basiliximab (82.4%); extracorporeal photopheresis (EP) (2.9%), Timoglobulin (8.8%) and others therapies (5.9%). Two patients (6%) obtained complete response (CR) and 10 patients (29%) partial response (PR). Global response (CR, PR) after second line (mainly basiliximab) showed better overall survival ( $P=0.009$ ). Third line: basiliximab (8.3%); EP (41.7%), mesenchymal cells (MSC) (8.3%), Ruxolitinib (16.7%) and others (24.9%). Ruxolitinib improve GVHD cutaneous and hepatic but not intestinal. The best results were achieved with EP (2 CR, 1 PR) and Basiliximab/MSC (1 PR, respectively). Only patients who achieved CR survived. Fourth line: MSC (50%)/Ruxolitinib (50%) does not improve the prognosis. No serious adverse effects were observed with MSC therapy, Basiliximab and EP. About 14% of patients showed CMV reactivation with Basiliximab. About 27 patients died (80%), 21 patients with early mortality (< 6 months) due to refractory aGVHD (40%) or secondary infections (60%). Overall survival at 6 months and 2 year was  $28 \pm 8\%$  and  $0\%$ , respectively. In multivariate analysis the main factor for TRM was the steroid-refractory vs steroid-sensitive (HR 2.00, 95% CI 0.91–4.39;  $P=0.083$ ) and was unfavorable the association of hepatic and intestinal aGVHD (HR 2.24, 95% CI 0.90–5.57;  $P=0.082$ ) No SR-aGVHD: 115 patients. TRM-100 was 18% ( $n=7$ ), mainly due to infection (71%). TRM-1 year was 37% ( $n=15$ ), mainly by GVHD (40%) and infections (40%). Median follow-up of 26 months, OS-6 months and 2 year were  $84 \pm 3\%/75 \pm 4\%$ , respectively. TRM was associated with not obtained CR/PR after second line ( $P=0.001$ ), no CR after third line ( $P=0.018$ ) and relapse of GVHD despite achieving CR initially ( $P=0.004$ ). In our series only the patients that obtained CR/PR after second-line or CR after third-line improved OS. The best results in SR-aGVHD were obtained with Basiliximab and extracorporeal photopheresis. TRM was associated with relapse of GVHD and advanced disease to the transplant. Randomized clinical trials are needed to assess different treatment modalities for SR-aGVHD.

[P226]



Disclosure of conflict of interest: None.

## P227 Previously published

## P228 Variable levels of suppressor function in granulocytic myeloid-derived suppressor cells in patients receiving extracorporeal photopheresis for chronic graft-versus host disease

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Extracorporeal photopheresis (ECP) is a therapy for steroid-refractory chronic graft versus host disease (cGVHD). Therapeutic response to ECP has been linked with a progressive increase in circulating granulocytic myeloid-derived suppressor cells (G-MDSC) in acute GVHD, but not in cGVHD<sup>1</sup>. Low density neutrophils (LDN) phenotypically resembling G-MDSC (putative G-MDSC) show marked flux in cGVHD patients receiving ECP, and a reduction in their frequency is associated with a sustained therapeutic response to ECP<sup>2</sup>. Recent data has identified Lectin-type oxidized LDL receptor-1 (LOX-1) as a specific marker of LDN with T-cell (Tc) suppressive activity<sup>3</sup>. Using this marker we have conducted a cross-sectional study to assess whether putative G-MDSCs in this patient cohort have suppressive activity. About 15 patients with steroid refractory or steroid-dependent cGVHD (mean treatment duration of 9 months) receiving ECP and 8 healthy controls were recruited. Patients had GVHD affecting skin (15/15), liver (3/15) and gut (2/15). PBMC were isolated and immunophenotyped by flow cytometry for markers of G-MDSCs (CD14<sup>-ve</sup>, CD16, CD66b, HLA-DR<sup>-ve</sup>, CD33<sup>int</sup>) and LOX-1 expression. Suppressive function was determined by measuring the inhibition of proliferation of anti-CD3/CD28-activated purified CD3 Tc from healthy donors by 4-day co-culture with G-MDSCs from patients. Statistical analysis was conducted using GraphPad 6. ECP patients had substantially greater frequencies of circulating putative G-MDSC than healthy controls ( $P < 0.0001$ ; median: 13% and IQR 2%–32% vs 0.2% and IQR 0.1%–0.6%, respectively). While there were substantially greater frequencies of circulating LOX-1<sup>+</sup> cells in PBMC from ECP patients than healthy controls ( $P < 0.0001$ ; median: 1.5% and IQR 0.39%–35% vs 0.053% and IQR 0.029%–0.062%, respectively), these were mainly the minority population within the putative G-MDSC fraction with no significant difference between ECP patients and healthy controls in the proportion of LOX-1<sup>+</sup> cells ( $29\% \pm 16\%$  vs  $21\% \pm 9\%$ , respectively). ECP had no significant effect on circulating putative G-MDSC frequency measured before and the day after treatment (median: 8.4% and IQR 4%–44% vs 16% and IQR 6%–25%;  $n=11$ , respectively) nor on LOX-1 frequency (median: 1% and IQR 0.29%–12% vs 2.8% and IQR 0.88%–7.3%;  $n=9$ , respectively). At a Tc:G-MDSC ratio of 1:1, isolated G-MDSCs from ECP patients suppressed CD3 Tc proliferation (mean  $\pm$  SD:  $52\% \pm 23\%$ ;  $n=14$ ). However, the potency of suppression was highly variable (min–max: 18–82%). The pattern of LOX-1 expression suggests that only a subset of putative G-MDSCs in ECP patients are suppressive and may explain why suppressive function in this cell fraction is so highly variable. However, the relatively high frequency of LOX-1 cells in this patient cohort might contribute to immunosuppression resulting in increased susceptibility to opportunistic infections.

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## Infectious complications

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Previously published

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### A retrospective study of central nervous system invasive fungal disease after allogeneic stem cell transplantation: risk factors, clinical characteristics and outcomes

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Cerebral invasive fungal disease (CNS-IFD) is a rare but fatal infectious disease. However, there are rare reports of CNS-IFD among allogeneic stem cell transplantation (HSCT) patients focus on risk factors, clinical characteristics and outcomes. During the period of January 2007 to June 2016, 3855 consecutive patients received their first allogeneic stem cell transplantation in Peking University Institute of Hematology. According to the revised EORTC/MSG criterion, 29 patients were diagnosed with CNS-IFD. Among those patients without CNS-IFD (3826 patients), CNS-IFD were matched in a 1:3 ratio for analyzing the risk factors of CNS-IFD. And among 594 (15.4%) patients who occurred pulmonary IFD without CNS involvement, 87 patients were selected as control group for analyzing the risk factors associated with involvement of CNS in pulmonary IFD. we selected the control group using a 1:3 ratio matched-pair method with the variates of (1) age; (2) sex; (3) underlying disease. We retrospectively reviewed 29 patients complicated with CNS-IFD after HSCT in our single center during a 10 years period. Most patients received haploidentical stem cell transplantation. The median onset time of CNS-IFD was 173 (24–972) after HSCT, and most (82.7%) of them have prior pulmonary IFD. The most frequent pathogen was *aspergillus*, while no *cryptococcus* and *candidas* were found. The most common clinical presentation was space-occupying symptoms and signs. Brain abscess were the most common imaging finding. Prior pulmonary IFD ( $P < 0.001$ , HR 62.746(95% CI, 14.28–275.27)) was the only risk factors associated with occurrence of CNS-IFD. While poor response at 6 weeks ( $P=0.045$ , HR 2.574 (95% confidence interval: 1.021–6.487)) was the only risk factor predicting the involvement of CNS in pulmonary IFD. The response (complete and partial response) at 12 weeks and last follow-up was 27.6% and 20.6%, respectively. The overall survival was 24.2% at the last follow-up with a median 289 (27–3341) days after transplantation. In conclusion, patients with pulmonary IFD had higher risk of CNS-IFD, especially in those with poor response after 6 weeks of treatment. And the prognosis of CNS-IFD was very poor after HSCT.

**Disclosure of conflict of interest:** None.

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### Activity of brincidofovir against adenovirus (ADV) infection in pediatric population after allogeneic stem cell transplantation: report of the French experience about 20 cases

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ADV may cause severe infections in HSCT recipients, especially from unrelated donors or cord blood particularly in pediatrics. Disseminated infections usually occur after digestive reactivations. At 3 Mo.post-HSCT, the incidence of ADV digestive infection and viremia in pediatric HSCT is about 30% and 15%,

respectively. Therapeutic strategies to control ADV infections are limited to the use of infusion of cidofovir (CDV) or *ex vivo* anti-ADV selected cytotoxic lymphocytes (CTL). However CDV is not labeled for ADV treatment, presents a renal toxicity and has shown limited efficacy. Specific-CTL remain difficult to produce. Brincidofovir (CMX001, BCV, Chimerix, USA) is an orally-available lipid conjugate of the nucleotide analog CDV that has demonstrated broad clinical antiviral activity against double-stranded DNA viruses (that is, herpes-, adeno-, orthopox- and polyomaviruses. The drug has an increased bioavailability compared to CDV and has shown encouraging results. We report here the results obtained with this compound in 20 patients treated in six centers from January 2015. There were 20 pts (8M/12F), median age at HSCT: 65 Mo. (15–202). HSCT indication was ALL in nine, PID in six, AML in two, FA in two and IBMF in one. Donor was 9/10 or 10/10 MUD in four and six pts, respectively; haplo-identical familial donor in 4; 5/6 or 6/6 unrelated CB in two and three pts, respectively; MSD in one. Stem cell source was BM for 11 pts, CB in 5 and PBSC in 4. Two pts underwent a second HSCT. Cond' regimen were MAC in 16 pts. All pts received either *ex vivo* or *in vivo* T-cell depletion. Three pts presented with ADV-disseminated disease, seven pts with blood + other site (throat, urine or stools) ADV infection, three with ADV-related gut disease, three with blood infection and three with gut infection. The remaining patient received BCV for JC viremia with fever. Median time for virus infection diagnosis was D20 post-HSCT (range: D-126 to D+300). About 13 pts experienced 24 other viral infection episodes after HSCT (CMV: 8; EBV: 5; BK: 5; HSV: 3; HHV6: 2; Influenzae: (1). About 12 pts received 1–6 injections of CDV prior to BCV treatment. One pt received specific-ADV CTL before BCV without efficacy. The reason to switch from CDV to BCV was uncontrolled ADV infection ( $n=11$ ) or CDV-induced renal failure ( $n=1$ ). Two additional pts experienced renal impairment after CDV. About 14 pts received 1–4 lines of immunosuppressive therapies (including ECP) in addition to calcineurin inhibitor at time of BCV therapy due to grade III and IV acute GvHD in seven and seven pts, respectively. Median ADV load at time of BCV initiation was 4.5log copies/ml (range: 3–9) in blood and 5log copies/ml (3.3–7.5) in stools. Median duration for BCV therapy was 3 weeks (range: 1–18). About seven pts with blood ADV infection or disseminated disease experienced ADV disappearance as well as four pts with gut disease or infection. Three of them experienced ADV infection relapse and received thereafter CDV, BCV or ADV-specific CTL. Five pts presented with grade 2–4 diarrhea during BCV treatment. About 13 were alive at end point where seven died from sepsis ( $n=2$ ), multi-organ failure ( $n=2$ ), GvHD ( $n=2$ ) and ADV disseminated infection ( $n=1$ ). Adenovirus infections occur often in immunocompromized pts receiving concomitant nephrotoxic drugs that may avoid CDV use. BCV appears as efficient therapy against adenovirus infection in such pediatric pts since here 13 out of 20 pts where alive after ADV infection and BCV treatment.

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### Actualization of CMV reactivation in hematopoietic stem cell transplant: haploidentical vs non-haploidentical donor transplant group (two-center experience)

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HLA-haploidentical hematopoietic stem cell transplant (haploHSCT) is a potentially curative strategy for patients lacking an HLA matched donor or a suitable unrelated donor. Due to an increase of haploHSCT, infectious complications seem to be growing up, particularly in terms of CMV reactivations;

**Table 1. Risk factors related to CMV reactivation. Multivariate analysis**

	CMV reactivation	<i>p</i> (Chi <sup>2</sup> )	OR (IC 95%); <i>p</i> (AMV)
<b>Haplo</b>	66.2%	0.000	11.83 (5.26-26.59) 0.000
<b>Grupo control</b>	29.8%		
<b>CMV R/D</b>	4%	0.000	2.37 (1.21-4.63) 0.011
-/-	19.2%		
-/+	54.2%		
+/-	38.9%		
+/+			
<b>Conditioning</b>	44.2%	0.102	1.98 (1.03-3.82) 0.04
<b>MAC</b>	35.6%		
<b>RIC</b>			
<b>GVHD agudo</b>	20.3%	0.000	3.10 (1.40-6.86) 0.005 13.23 (4.81-36.35) 0.000
<b>No GVHD</b>	37.7%		
<b>I-II</b>	64.4%		
<b>III-IV</b>			
<b>CorticoR GVHDa</b>	68.8%	0.002	

**Disclosure of conflict of interest:** None

moreover no direct comparison with non haploidentical (NH) donors have been reported. Between August 2012 and June 2016, 284 patients received an HSCT in Salamanca and Marqués de Valdecilla University Hospitals. In this study we retrospectively analyzed CMV reactivation determined by PCR and response to pre-emptive therapy in patients receiving an haplo ( $n=68$ , 24%) comparing them with a control group of non haploHSCT (110 MRD and 106 MUD) ( $n=216$ , 76%). Median age was 52 years (range: 16–71), 56 for haploHSCT and 52 for control group. Conditioning regimen was myeloablative (MAC) in 33.5% and reduced intensity (RIC) in 66.5%. HaploHSCT characteristics: haplo conditioning was Fludarabine (30 mg/m<sup>2</sup> or 50 mg/m<sup>2</sup> × 4 days in RIC or MA regimen) and Busulfan (3.2 mg/kg × 2 in RIC or 3 days in MA) (19.1% MAC, and 80.9% RIC). Cyclophosphamide-post was used for GVHD prophylaxis in 94%. Median of days to reach more than 500 × 10<sup>9</sup> granulocytes and more than 20 × 10<sup>9</sup> platelets were 17 (13–31) and 22 (0–103), respectively. Incidence of acute GVHD was 70% (grade I–II 64.2%, and III–IV 5.7%), with two steroid-refractory cases. CMV reactivation: 66.2% of haploHSCT patients presented CMV reactivation, vs 29.8% in control group ( $P=0.000$ ). Median number of CMV reactivation episodes was 1 in both groups. Median time to CMV PCR detection was 35 days (4–70) and 40 (0–186) in haploHSCT and control group respectively ( $P=0.042$ ). Average maximum CMV IU by PCR was 17.499 in haplo vs 8.206 in the control group ( $P=0.035$ ). First antiviral pre-emptive therapy (Valganciclovir in 65.7%) was effective in 82% in haploHSCT vs 65% in control group ( $P=0.064$ ). Main reason for antiviral treatment switch was failure in CMV IU reduction, and Foscarnet was the most used therapy in refractory cases. Twenty patients developed CMV disease (5 in haplo and 15 in control group) (GI disease 90% and pulmonary disease 10% in both groups). In a multivariate Cox-regression model, receiving an HaploHSCT, serological CMV status (positive patient/negative donor), MAC regimen and development of acute GVHD grade I/II or grade II/IV were variables associated with a higher risk of CMV reactivation. Based on these results, haploHSCT is associated with a higher CMV reactivation compared to non-haploHSCT,

despite a lower incidence of all other risk factors as aGVHD or MAC in the haplo group. Although it is not statistically significant, response to pre-emptive therapy is higher in haploHSCT and no differences in CMV disease were observed. **Disclosure of conflict of interest:** None.

**P233****Allogeneic stem cell transplantation in patients with HIV infection**

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Although a number of patients with HIV infection and hematological disease have successfully undergone allogeneic HSCT together with combination anti-retroviral therapy (cART), short and long-term outcomes remain not well known. We report the Spanish experience treating HIV-infected adult patients with high-risk hematological malignancies with allogeneic HSCT. We retrospectively reviewed 17 HIV-positive patients who received allogeneic HSCT in three institutions in Spain within GETH (Grupo Español de Trasplante Hematopoyético y Terapia Celular). Seventeen patients have been transplanted between 1999 and 2015. Median age was 44 (30–57), 82% male. Diagnosis and transplant characteristics are summarized in Table 1. Cumulative incidence of neutrophil and platelet engraftment were 88% at 30 days (median 15 days), and 76% at 60 days (median 13 days), respectively. With a median follow-up of 42 months (22–87), OS and EFS were 35%. TRM was 17% at 12 months and 32% at 36 months. Grade II–IV aGVHD rate was 41%, and moderate/severe cGVHD rate was 41%. All patients received cART. Two patients showed severe toxicity related to interaction of immunosuppressive

**Table 1**

Characteristic	n=17
Diagnosis, n (%)	
NHL/HL	8 (47) / 2 (12)
AML/ALL	4 (23) / 1 (6)
Other	2 (12)
Donor type, n (%)	
HLA-identical sibling / unrelated	10 (59) / 2 (12)
HLA-haploidentical	4 (23)
Haplo-Cord*	1 (6)
Conditioning regimen, n (%)	MAC=6 (35)/ RIC=11 (65)

\*Haplo-Cord: Single cord blood supported by CD34+ cells from third party HLA-mismatched donor

drugs and protease inhibitors. About 76% of patients showed infectious complications. Viral infections were the most frequent cause: CMV (9), BK (2), ADV (1), HHV-1 (2), HCV (1), HHV-8 (1), parainfluenza (1). Two patients had invasive aspergillosis and one patient presented disseminated tuberculosis. Causes of death were: relapse (4), infection (3), GVHD (2) and toxicity (1). All surviving patients showed undetectable HIV load after HSCT. Allogeneic HSCT is an effective therapy for high-risk haematological malignancies in patients with HIV infection, and long-term HIV suppression with cART is feasible. However, interactions between immunosuppressive agents and anti-retroviral drugs, high rates of significant GVHD, and frequent infectious complications account for a complex procedure in this population. Selected HIV-infected patients with hematologic malignancies should be considered for allo-HSCT when indicated, in experienced centers.

**Disclosure of conflict of interest:** None.

#### P234

##### **Analysis of risk factors influencing course and outcome of clostridium difficile infection in children undergoing hematopoietic stem cell transplantation**

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Clostridium difficile infection (CDI) is one of the most common causes of nosocomial infectious diarrhea in Europe and USA, which results in high morbidity and mortality among hospitalized patients. Allogenic hematopoietic stem cell transplant (HSCT) recipients remain at high risk for CDI. Incidence rate ranges from 2 to 27%. Numerous risk factors

including acute graft-versus-host disease (aGVHD), HLA matching status, conditioning-intensity, use of total body irradiation (TBI) may play an important role in the course of CDI in these patients. The aim of this study was to evaluate the prevalence of CDI in children, and to assess the influence of such factors as gender, age, diagnosis, HLA matching status, conditioning-intensity, use TBI-containing regimen, source of graft (bone marrow/BM/ vs peripheral blood/PB/or aGVHD on course, duration of treatment and outcome in children undergoing HSCT. Between 2014 and 2015 a total of 342 HSCTs were performed in five Polish Pediatric Transplant Centers, including 267 allogeneic and 75 autologous. All patients were followed up to 6 months post HSCT. We analyzed retrospectively 29 episodes of CDI infection in the group of 342 children. Twenty-one of 29 children were diagnosed with hematological malignancies: Acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), myelodysplastic syndrome (MDS), two were diagnosed with severe aplastic anemia (SAA), one with chronic granulomatous disease (CGD) and 5 of them—with solid tumors. The median age was 9.3 years (range: 2.5–19.0 years). Majority of patients underwent myeloablative conditioning protocol (24/29). In allogeneic setting 21/24 patients underwent MUD-HSCT, 2/24 pts MSD-HSCT and one patient was given a haploidentical PBSCT. In this series, in 7 out of 24 cases BM was a transplant source, and PB in 17 out of 24. CDI was defined as having diarrhea that tested positive for C. difficile by PCR, cytotoxin assay, or dual enzyme immunoassays. Kruskal-Wallis test, Wilcoxon test and  $\chi^2$ -test were used to estimate the influence of risk factors on severity of disease, duration of treatment and outcome. We observed 29 episodes of CDI (8.5 %) in HSCT recipients: in 24 allotransplant recipients (8.9% of all transplants) and in 5 autotransplant recipients (6.7% of all auto-HSCT). Nine patients responded to therapy with metronidazole, seven patients responded to vancomycin alone, and in two patients rifaximine was administered. Six children required adding second drug: vancomycin or metronidazole, five patients were not given any medications. There was no significant correlation between such factors as diagnosis, gender, age, conditioning regimen, HLA matching, aGVHD and severity of disease, and duration of treatment. Recurrence rate was difficult to assess due to lack of data. We observed three deaths. One of them was connected with CDI. There was one 17-year-old boy with SAA (MUD-PBSCT, HLA 10/10) with no aGVHD. The other two deaths were due to progression of

disease. CDI occurred in nearly 9% of pediatric patients undergoing HSCT, surprisingly often in autologous HSCT too (6.7%). Almost all patients experienced mild CDI with adequate response to antibiotic therapy. CDI is a rare cause of death among transplant recipients.

**Disclosure of conflict of interest:** None.

### P235

#### Antifungal prophylaxis in high-risk paediatric patients with haematological malignancies: a monocentric experience

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The choice of antifungal prophylaxis in high risk paediatric haematological patients (according to the latest ECIL6/SEIFEM guidelines) remains an open question. A recent retrospective survey from Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) showed that, in these patient categories, the only variable which significantly impacted on invasive fungal infection (IFI) occurrence was the presence or not of antifungal prophylaxis at the IFI onset (unpublished data). From January 2012, in our Pediatric Hematology Oncology Unit, 31 allogeneic hematopoietic stem cell transplantations (HSCT) were performed (median age: 10 years; range: 6 months–23 years), mainly for acute leukaemia (median follow-up: 24 months; range: 4–50). Patients received liposomal amphotericin B ( $n=20$ ), micafungin ( $n=10$ ), or fluconazole ( $n=1$ ) as primary antifungal prophylaxis until neutrophil recovery ( $>1 \times 10^9/L$ ). Seven patients developed acute GvHD (22%) which evolved in GvHD in 5 (16%). As outpatients, they continued with posaconazole ( $n=17$ ), voriconazole ( $n=4$ ), or micafungin ( $n=10$ ) until CD4+T-cell recovery ( $>200/cmm$ ) or GvHD immune suppressive prophylaxis/treatment withdrawn. During the last year, according to ECIL6/SEIFEM guidelines<sup>1</sup>, we administered primary antifungal prophylaxis also to 10/12 high risk (HR) acute leukaemia patients. Two patients with AML were treated with posaconazole, four patients with HR-ALL received micafungin, four relapsed ALL patients received micafungin ( $n=2$ ), or liposomal amphotericin B ( $n=1$ ), or posaconazole ( $n=1$ ). One AML patient was then transplanted; all relapsed patients are waiting for transplant. No differences were observed in terms of breakthrough proven/probable (PP)-IFI incidence, according to antifungal prophylaxis in the various patient groups. In particular, in the early phase, we observed a PP-IFI incidence of 10% in both treatment arms (micafungin vs liposomal amphotericin B,  $P=NS$ ). In the late phase, we observed 1 case of PP-IFI who were receiving posaconazole as prophylaxis. Overall survival (OS) was 96%, with 4% mortality rate. In HR leukaemia patient group, we observed PP-IFI in the only two patients who were not receiving any antifungal prophylaxis at the IFI onset. Antifungal prophylaxis is strongly recommended in paediatric patients with haematological malignancies who are at high risk of IFI. The choice of antifungal drug depends on the treatment phase, drug interactions (particularly for azoles), patient compliance and clinical conditions which interfere with intestinal absorption. In our experience, as no differences were observed in term of efficacy, micafungin resulted the best choice in terms of tolerability, toxicity, compliance and cost saving.

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### P236

#### Antifungal prophylaxis with micafungin and bridging to inhaled liposomal amphotericin B after engraftment in patients undergoing allogeneic hematopoietic stem cell transplantation

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Micafungin is an effective antifungal for prophylaxis, active against *Candida* spp. including those resistant to other antifungals (*C. Glabrata* and *C. krusei*) and also active against *Aspergillus* spp. Guidelines focused on antifungal prophylaxis, recommend its use during preengraftment and early post-engraftment period in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. Moreover, its profile of low drug interactions and side effects, makes it a suitable alternative for patients who need concomitant treatments, present hepatic insufficiency and in those who do not tolerate oral drug administration. The addition of inhaled liposomal amphotericin B (LAmB) after engraftment, provides an alternative way to effectively prevent mold infections, that are acquired mainly by inhalation. Inhaled LAmB has good tolerance with absence of drug interactions and low toxicity. The aim of this study was to describe the experience in the HSCT unit of the University Hospital of Salamanca with micafungin and LAmB as primary prophylaxis in patients undergoing allo-HSCT with reduced intensity conditioning (RIC) and graft-versus-host disease (GVHD) prophylaxis with tacrolimus and sirolimus. Thus evaluating efficacy and tolerability in our population. Retrospective observational study from January 2013 to August 2016, including all adult patients undergoing allo-HSCT with RIC and GVHD prophylaxis with tacrolimus and sirolimus, in whom an azole derivative is not indicated, due to drug interactions. Therefore received prophylaxis with micafungin during the preengraftment period and bridging to LAmB after engraftment at discharge, and continuing it during the first 100 days post-transplant. Data from 106 patients from our HSCT unit. Ten (9.4%) patients who had invasive fungal infection before undergoing allo-HSCT, and 16 (14.8%) patients who received prophylaxis with drugs other than micafungin-LAmB were excluded. Underlying disease was grouped by leukemia in 44 (41.5%) patients, lymphoma in 23 (21.7%), myelodysplastic syndromes in 18 (17%), multiple myeloma in 11 (10.4%) and other diseases in 10 (9.3%) patients. Eighty patients underwent peripheral blood allo-HSCT, of whom were related donor in 40 (50%) patients and unrelated donor in 40 (50%). Prophylaxis with micafungin in 80 (75.5%) patients, dose of 50 mg per day, with a mean of 19 days ( $\pm 6$  days) with postengraftment bridging with LAmB 24 mg weekly, continuing it during the 100 days post-transplant. Days of neutropenia during preengraftment,  $<14$  days in 24 (31.2%) patients, 14–28 days in 49 (63.7%), more than 28 days in 4 (5.2%). During follow-up there were three cases (3.8%), two catheter related candida infection, and one esophageal candidiasis. There were no reported aspergillosis cases (possible, probable or proven), according to the European Organization for Research and Treatment of Cancer (EORTC) criteria. Finally, prophylaxis with micafungin and inhaled LAmB, was considered an effective and safe strategy in 77 (96.3%) patients, with no side effects reported. According to our experience with micafungin and the addition of inhaled liposomal amphotericin B, the results indicate that, this is an appropriate alternative for antifungal prophylaxis, in patients undergoing allo-HSCT, because of their efficacy, few side effects and drug interactions.

**Disclosure of conflict of interest:** None.

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**Antifungal prophylaxis with nebulized liposomal amphotericin B and fluconazole is effective and safe in the early phase of allogeneic hematopoietic stem cell transplantation**

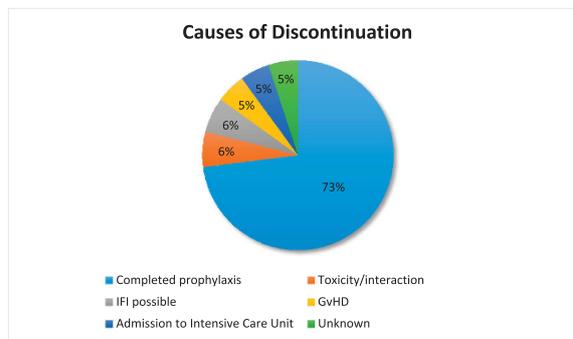
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Recipients of allogeneic hematopoietic cell transplantation (alloHCT) are at high risk of developing invasive fungal infections (IFI). In the early phase (< 100 days) after alloHCT, the use of antimold prophylaxis has been generalized, although there is no consensus on the best therapeutic strategy. The use of nebulized liposomal amphotericin B and fluconazole has been shown to be effective, safe and associated with low economic costs in lung transplantation<sup>1</sup>. However, the use of this prophylactic strategy in the early phase of alloHCT setting has not been evaluated. We included all consecutive patients who received their first alloHCT in our center from January 2013 to August 2016 and who underwent antifungal prophylaxis according to the prospective AMBINEB protocol (nebulized liposomal amphotericin B 24 mg administered three times per week as loading dose and once per week and fluconazole 200 mg per day until day +90). Patients with a previous IFI were excluded. Patients with graft-versus-host disease (GvHD) receiving high dose corticosteroids were allowed to be changed to voriconazole or posaconazole at physician's discretion. The primary objective of the study was the incidence of IFI at day +180. The secondary objectives were to assess adherence and toxicity of the AMBINEB protocol. Only cases with proven or probable IFI according to EORTC-MSG criteria were considered. A multidisciplinary team of experts in hematology, infectious diseases, microbiology and radiology prospectively evaluated and categorized each case. We included 102 patients with a median age of 50 years (range: 18–70) and a median follow-up for survivor of 14 months (range: 2–47). Patients received alloHCT mainly for acute leukemia (55%), non-Hodgkin lymphoma (16%) and myelodysplastic syndrome (12%). Patients received alloHCT from HLA unrelated (59%) or related donors (28%) mostly using a reduced intensity conditioning (57%). Graft-versus-host disease (GvHD) prophylaxis was performed with calcineurin inhibitors mainly in combination with sirolimus (57%) or methotrexate (17%). After the comprehensive review, only one case of proven or probable IFI at day +180 was diagnosed. Prophylaxis with AMBINEB was completed in 75 patients (74%) while 27 (26%) stopped the treatment. The most frequent causes of discontinuation were possible IFI (6%), GvHD (5%), admission in intensive care unit (5%) and toxicity (6%) (Figure). Ninety-four patients (92%) did not have adverse events associated with AMBINEB. Eight patients presented organ toxicity which was at least partially attributed to AMBINEB, including gastrointestinal symptoms (n=4), liver function test abnormalities related to fluconazole (n=3) and cough (n=1). Of the 27 patients who discontinued AMBINEB, 22 (81%) were switched to other antifungal drugs including (echinocandins (12, 54%) posaconazole (4, 18%), voriconazole (2, 9%) or others (4, 18%)). Overall survival and non-relapse mortality of all patients at median follow-up were 64% (95% CI 58–69) and 25% (95% CI 20–30), respectively. The combination of nebulized ambisome and fluconazole is effective in preventing IFI in the early phase of allo-HCT and is associated with high adherence and low toxicity.

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[P237]



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**Bacterial isolates and bacteremias following autologous peripheral stem cell transplantation (AP SCT) in multiple myeloma (MM) and diffuse large B cell non-Hodgkin's lymphoma (DLBCL): a single center experience**

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Neutropenia-related infections is a common complication of AP SCT in patients (pts) with MM and DLBCL. Our study aims are to: (1) assess antibacterial susceptibility patterns of isolated organisms, to guide antibiotic prophylaxis (2) identify the epidemiology of bacteremia with susceptibility patterns to direct empiric therapy of febrile neutropenia (3) assess the interval between the occurrence of neutropenia and the isolation of resistant bacteremia to identify the best timing to start prophylaxis (4) identify contributing factors for the development of bacteremia and mortality. Our retrospective study included 191 adult pts who underwent AP SCT for MM and DLBCL between 2005 and 2015. We recorded the following: age, gender, basic illness, comorbidities, number of CD34 + cells infused, a central venous catheter, duration of neutropenia, diarrhea and mucositis, mechanical ventilation, positive bacterial cultures with susceptibility profiles and history of broad-spectrum antibiotic intake for more than 72 h for the past 3 months on hospital setting, and mortality. Statistical analysis was carried out using SPSS (version 19). About 102 isolates were obtained: urine (46.5%), blood (28.5%), sputum (9%), wound (7%), venous catheter (7%) and stool (2%). Gram-negative (GN) species were predominant (73.5%) with *E. coli* (32.5%), *Klebsiella* (K) (11%) and *Pseudomonas* (Pseudo) (11%). Isolates sensitive to third generation cephalosporins (3GC) represented 83% of the Enterobacteriaceae (Entero) including 78% in *E. coli* and 73% in K. All Entero isolated were susceptible to carbapenems (carba), piperacillin/tazobactam (PIP/TAZ), amikacin and ciprofloxacin (cipro). All Pseudo (n=11) and Acinetobacter (n=4) isolates were susceptible to carba, PIP/TAZ, amikacin, cipro, colistin and

tigecycline. As for Gram-positive (GP) bacteria (26.5%), coagulase negative staphylococci (CNS) were predominant (14%). Oxacillin susceptibility reached 29% and two isolates methicillin resistant *S. aureus* were identified. All GP were susceptible to glycopeptides. A total of 29 bacterial isolates were identified (29 episodes of bacteremia) from 24 pts. GN were predominant (72.5%) with *E. coli* being most common (24%). All GN were susceptible to 3GC, carba, PIP/TAZ, amikacin and cipro. As for GP (27.5%), CNS predominated (24%) including 28% oxacillin-susceptible causing seven episodes of bacteremia with six central line-associated. No glycopeptide resistance was identified. None of the clinical features and pts' characteristics reached statistical significance as risk factor for bacteremia. However, the need for mechanical ventilation and mortality were higher in bacteremic vs non-bacteremic pts (16.7% vs 3%,  $P=0.004$ , and 12.5% vs 3%,  $P=0.036$ , respectively). All bacteremic episodes occurred after developing neutropenia (median = 2.5 days, range: 1–9) except for one case of CLABSI caused by *E. coli* occurring 1 day before neutropenia. PIP/TAZ was prescribed in 21% of bacteremia episodes followed by quinolones (11%) and carbapenems (4%). No previous use of third and fourth generation cephalosporins was observed. We recommend quinolone prophylaxis in APSCT pts. For empiric therapy, antibiotics recommended by international guidelines including, cefepime and PIP/TAZ still fit. Thus, we could spare the use of carba and other last-resort antibiotics to other conditions. We also recommend continuous surveillance of resistance.

**Disclosure of conflict of interest:** None.

#### P239

##### Previously published

#### P240

##### **Blood culture number is related to microbiological documentation rate of febrile episodes in patients undergoing autologous stem cell transplantation: single-centre experience in 66 consecutive procedures**

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Fever is an almost universal complication in patients undergoing autologous stem cell transplantation (ASCT), however, microbiological documentation is only achieved in 20–30% of such febrile episodes (FE).<sup>1</sup> This low diagnostic efficiency makes epidemiological assessment in transplant units difficult, and may lead to a suboptimal empirical treatment. We have studied the utility of strict blood culture (BC) extraction as a mechanism to improve microbiological documentation of FE in these patients. We conducted a retrospective study over 66 consecutive ASCT performed in our centre between June 2015 and May 2016 (1 year). About 33 patients were male and 27 female, with age between 27 and 72 years (mean 57.8). Diagnosis was 4 Hodgkin Lymphoma, 27 non Hodgkin lymphoma and 35 multiple myeloma. ASCTs were performed in reverse isolation conditions, in rooms equipped with HEPA and Pall filters. Prophylaxis against herpes virus and *P. jirovecii* with acyclovir and pentamidine was used. No prophylaxis against Gram negative bacteria or filamentous fungi was performed. BC were extracted at the beginning of every FE and every 48–72 h if fever persisted (or more frequently, following clinical criteria). Blood samples from intravascular devices and peripheral blood were collected in two BACTEC bottles each (for aerobic and anaerobic microorganisms). Complementary diagnostic techniques and empiric antibiotic therapy were performed following our institution's guidelines. FE were classified in microbiologically documented infections (MDI), clinically documented infection (CDI) and fever of unknown origin (FUO) following HIS criteria.<sup>2</sup> 85 FE were studied (average 1.28 FE per patient, 6.04 days of fever

duration per FE). About 28% of FE were classified as MDI, 26% as CDI and 46% as FUO. MDIs were caused by gram positive bacteria (56%), Gram negative bacteria (26%) and polymicrobial infections (17%). No viral or fungal infections were observed. An average of 7.2 BC per FE and 7.6 per patient were extracted. The proportion of FE classified as MDI was related to the number of blood cultures extracted during the episode. Only 8% of FE with three or less BC extracted were classified as MDI, 21% if 4–6 BC were extracted, and 55% if 7–9 BC were extracted ( $P < 0.05$ ). No significant difference in proportion of MDI classified FE between the extraction of 10 or more BC and 7–9. All patients were discarded in good clinical conditions. According to our experience, a strict 7–9 blood culture extraction is related to a high rate of microbiological documentation of febrile episodes. Moreover, we have not observed the rise in Gram negative bacteria reported by other studies and gram positive cocci persist as the main infection cause in our centre.

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**Disclosure of conflict of interest:** None.

#### P241

##### **Candida is an emerging pathogen beyond the neutropenic period of allogeneic hematopoietic cell transplantation**

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*Candida* which has been traditionally related to duration of neutropenia, emerges as a pathogen beyond the aplastic period in allogeneic haematopoietic cell transplantation (alloHCT). In the setting of alternative transplants and aggressive immunosuppressive therapy, these infections are a challenging problem. There is scarcity of data regarding the significance of breakthrough candidaemia in alloHCT. To that end, we aimed to determine the incidence, clinical and microbiological characteristics and outcome of candidaemia in alloHCT recipients. We studied consecutive alloHCT recipients from January 2014 to June 2016. Blood cultures were obtained from peripheral vein or central venous catheters (CVCs) routinely and on febrile patients. Well-known risk factors for candidaemia were studied: neutropenia, type of transplant, moderate to severe graft-versus-host disease (GVHD) and co-existing infections. Among 108 alloHCT recipients, we identified seven patients with candidaemia: five post matched unrelated (four myeloablative and one reduced intensity conditioning) and two post haploidentical transplant. In median time of 3.5(1.3–8) months, 20 episodes of candidaemia were noted, despite antifungal prophylaxis with echinocandins or azoles. Infections with non-albicans *Candida* spp. occurred more frequently (19/20) and *C. parapsilosis* was the predominant microorganism (11/19). Other species were isolated: *C. famata* (5), *C. krusei* (2) and *C. haemulonii* (1). All *Candida* spp. isolates were phenotypically susceptible to antifungal agents already administered to patients. There was no resistance to echinocandins indicated by minimum inhibitory concentrations (MICs). All patients had severe acute or late-onset GVHD with intestinal involvement and CVCs prior to candidaemia. Although CVCs were removed in 7/7 and patients were treated with echinocandins, new CVCs were re-contaminated in 4/7 with the same or other species. All patients presented well

known risk factors for candidaemia (use of broad spectrum antibiotics due to severe bacterial infections, total parenteral nutrition due malnutrition, long-term high-dose corticosteroids and other immunosuppression), but no neutropenia. One patient survived, whereas five patients succumbed to GVHD and multi-organ failure and one patient to sepsis due to bacteremia. Candidaemia was observed in non-neutropenic patients with aGVHD and CVCs on antifungal prophylaxis, despite difficulties in diagnosis due to poor sensitivity of blood cultures. The epidemiology of candidaemia has changed in the last decade and its risk is more diverse and complex. The irreversible intestinal GVHD lesions might be the main source of Candida in patients receiving antifungal prophylaxis. Our data show that candidemia remains an important issue in profoundly immunosuppressed patients contributing to excessive morbidity.

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**Disclosure of conflict of interest:** None.

**P242**

**Ciprofloxacin prophylaxis for the prevention of neutropenic sepsis following allogeneic stem cell transplantation**

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Following the 2012 National Institute of Clinical Excellence (NICE) clinical guidelines on neutropenic sepsis, the Royal Marsden NHS Foundation Trust introduced the use of oral ciprofloxacin prophylaxis (500 mg 12 hourly) in all patients undergoing haematopoietic stem cell transplantation in January 2016. We conducted a retrospective study of all patients undergoing allogeneic stem cell transplantation between the period of January 2015 and October 2016. Our aim was to compare the rate of neutropenic sepsis, defined as fever of >38 °C and a neutrophil count of <0.5 × 10<sup>9</sup>/L, before and after the introduction of ciprofloxacin prophylaxis. One hundred and eight adult patients, of which 60 had acute myeloid leukaemia, 17 had acute lymphoblastic leukaemia,

12 had lymphoma and 19 had other haematological malignancies, were identified through our admission database. Of these 108 patients, 48 received oral ciprofloxacin during their neutropenic phase. The median duration of neutropenia was 13 days in both the no-prophylaxis and ciprofloxacin groups. There was a significant reduction in the rate of neutropenic sepsis from 88.3% (53/60) in the no-prophylaxis group to 68.8% (33/48) in the ciprofloxacin group (*P* = 0.012). Prolonged infection, suggested by the use of broad-spectrum antibiotic treatment for more than 10 days, was more common in the group which did not receive prior ciprofloxacin prophylaxis (45.0% vs 20.8%, *P* = 0.009). Rate of intensive care admission (15.0% vs 0.0%, *P* = 0.005) was also reduced by the use of ciprofloxacin. However, there was no significant difference in the length of stay (mean of 28 vs 25 days, *P* = 0.187), or in the 30-day infection-related readmission rate (17.9% vs 13.3%, *P* = 0.536) between the two groups. In terms of the cause of neutropenic sepsis, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the most common bacteria isolated from cultures in the no-prophylaxis group. Eighty percent of these organisms showed sensitivity to ciprofloxacin. In the ciprofloxacin group, *Staphylococcus epidermidis* was the most frequently found bacteria. With regards to treatment related adverse effects, none of the patients who received ciprofloxacin prophylaxis developed *Clostridium difficile* diarrhoea. In conclusion, ciprofloxacin is still an effective antibacterial prophylaxis during neutropenia following allogeneic stem cell transplantation. Clinicians should have a high suspicion of a Gram-positive infection in patients who develop neutropenic sepsis on ciprofloxacin prophylaxis.

**Disclosure of conflict of interest:** None.

**P243**

**Clinical outcomes of fecal microbiota transplantation for overcoming multidrug resistant infection complications after haploidentical hematopoietic stem cell transplantation**

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Fecal microbiota transplantation (FMT) is a novel method of treatment of intestinal microbiota malfunction usually due to *Clostridium difficile* infection (1). In the case of haploidentical

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**Table 1: Patient characteristics and FMT outcome**

Patient	FMT number	Diarrhea before FMT, months	<i>Clostridium difficile</i> toxin B before FMT	<i>Clostridium difficile</i> toxin B after FMT	Calprotectin before FMT, mcg/g	Calprotectin after FMT, mcg/g	Time of response, weeks
1	2	6	Positive	Negative	290	10	2
2	1	1	Positive	Positive	44	83	2
3	3	3	Positive	Negative	183	61	4

FMT may be safe and effective method to overcome multidrug resistance and treat infection complications in immunodeficiency patients after haploHSCT. Keywords. Fecal microbiota transplantation, haploidentical HSCT.

hematopoietic stem cell transplantation (haploHSCT) to cure leukemia, malignancy and some inherited diseases, different additional reasons interfere microbiota metabolism and integrity. Among them are radiation and chemotherapy, mucositis, infection and graft versus host disease (GVHD). The curative mechanism of FMT is based on the ability of donor intestinal microbiota to substitute and to provide all necessary functions of altered patient's microbiota. Three patients (3, 10 and 28 years old) after haploHSCT, who observed pseudomembranous colitis (toxin B-positive) as GVHD of intestine outcome, were enrolled to the study and performed FMT. Relatives (mother, father and brother) were used as microbiota donors. Donor and patient examination have included routine clinical and biochemistry laboratory data, microbiota cultural methods, PCR of most common intestinal microorganisms. Additional for patient-level of fecal calprotectin by ELISA was tested, identification of drug resistant bacteria and histology of intestine were made. Patient's preparation for FMT included-probiotic (inulin) administration 72 h prior procedure, discontinuation of all antibiotics 24 h prior procedure and antiemetics (5HT<sub>3</sub>-agonist), prokinetics and proton pump inhibitor. Delivery of donor's microbiota was performed in two consecutive steps under total intravenous anesthesia: with esophagogastroduodenoscopy-to the duodenum; with colonoscopy-to the caecum. All patients observed complete clinical response in 14-28 days after FMT (Table 1). In 10 days we have revealed significant quantitative and qualitative changes in microbiota composition, which was matched to donor's microbiota. In 35 days after FMT we identify microbiota changes in oropharynx and urogenital tract similar to donor microbiota. This leads to substitution of multidrug resistant *Klebsiella pneumoniae* strains by drug sensitive microorganisms and helps to treat severe infection complications after haploHSCT.

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**Disclosure of conflict of interest:** None.

#### P244

##### CMV, EBV and HHV-6 reactivation after allogeneic stem cell transplantation does not linked to viral contamination of apheresis platelet concentrates

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Recent works showed high proportion of CMV reactivation in patients after haploidentical HSCT (A. Laberko *et al.* EBMT 2016 #0140, M. Kerbauy *et al.*, ASH 2016 #2216). It has raised the question whether it could be or not due to a contamination of blood products by donors' human herpes viruses. In order to find out whether CMV, EBV and HHV-6 could be transfused or not with apheresis platelet concentrates we conducted the current study. About 41 pediatric cases after allogeneic HSCT, performed in our institution from August 2007 till September 2016. Among them there were 22 boys and 19 girls with median age of 7.4 years (range: 1.0-17.4). 30 patients had malignant and 11 non-malignant disease. MSD was used in 16 cases, MUD (10/10 matched) in 14, haploidentical donor in 11. CMV status assessed by serology of donor (D)-recipient (R) pairs were as follows: D+/R+ in 27 cases, D-/R- in 1 case, D+/R- in 3, D-/R+ in 10. CMV preemptive therapy was administered in 34 patients. Platelet aphereses were carried out in 83 donors (50 males and 33 females with median age 38.5 years) using Haemonetics instrument with simultaneous leucoreduction. Quantitative detection of CMV, EBV and HHV-6 DNA was performed by multiplex real-time quantitative PCR kit (Interlabservice, Russia) in donors' whole PB, plasma and platelet aphereses at the time of platelet collections. Viral load in HSCT recipients was monitored weekly after HSCT and 7 days before HSCT by the same PCR kit. Lower limit of

detection (LLOD) of the applied kit for all viruses was 500 copies/ml. In specimens of platelet donors we additionally performed ultra-sensitive PCR with LLOD 100 copies/ml. Only one patient (2.4%) was CMV-positive by PCR prior to HSCT. CMV reactivation after HSCT  $\geq 500$  copies/ml was noted in whole PB of 9 patients (22.0%) with median time of 41 days (range: 0-71). Donor source in CMV-reactivated patients was as follows (3 MUD, 1 MSD, 3 haplo). CMV viremia  $\geq 1000$  copies per ml was detected in seven patients (17.1%). CMV disease was found in five cases (12.2%). None of patients were positive by PCR for EBV or HHV-6 prior to HSCT. EBV reactivation  $\geq 500$  copies/ml was found in six cases (14.6%),  $\geq 1000$  copies/ml in 5 (12.2%) with median time of 27 days (range: 20-56). No signs of PTLD or other EBV-dependent clinical symptoms were observed. HHV-6 level after HSCT  $\geq 500$  copies/ml was detected in 17 patients (41.5%),  $\geq 1000$  copies/ml in 11 ones (26.8%) with median time of reactivation 19 days (range: 13-36). HHV-6 disease was observed in one patient. None of platelet donors were CMV-positive in plasma, whole blood or platelet aphereses products. EBV  $\geq 500$  copies/ml was detected in whole PB specimens of five platelet donors (6.0%). Application of ultra-sensitive PCR revealed low level of EBV-viremia in additional 11 PB cases with median EBV level 260 copies/ml (range: 115-410). None of platelet donors have any clinical signs of EBV disease. There is no any EBV-positive case among platelet concentrate specimens. In two cases low levels of HHV-6 was found in a whole PB (110 and 180 copies/ml). None of HHV-6-positive case was observed among plasma and platelet concentrate specimens. Despite high incidence of CMV, EBV and HHV-6 reactivation after HSCT in pediatric patients we could not show that source of viral reactivation was contamination of platelet apheresis products by donor-derived herpes viruses.

**Disclosure of conflict of interest:** None.

#### P245

##### Previously published

#### P246

##### Conventional respiratory virus infections after stem cell transplantation: risk factors, outcomes and changes over time

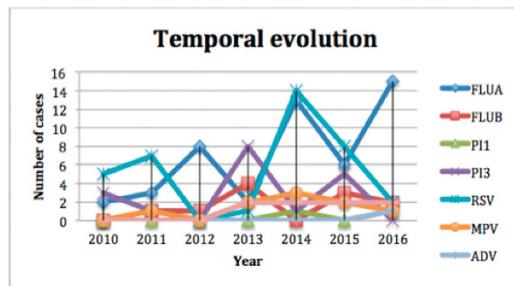
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Conventional respiratory virus (CRV) infections are known to be major causes of morbi-mortality after stem cell transplantation (SCT) due to the increased risk of progression to lower respiratory tract infection (LRTI) in this setting. Risk of developing severe LRTI is mostly related to factors specific to the patient and the underlying disease, although the intrinsic virulence of CRVs may also determine their outcomes. We conducted a single-center retrospective study including all adult SCT recipients who had CRV disease (defined as patients with symptomatic respiratory disease and CRV identification) during a 7-year period (2009-2016) with the main objective of evaluating epidemiological changes over time and their association with infection outcomes. During the study period 137 episodes of CRV disease were diagnosed in 104 patients (median age: 48 years, 58% male, 49% AML or MDS as baseline disease). 83 patients (80%) received an allogeneic-SCT (allo-SCT) (30% had a prior SCT) and 21 (20%) an autologous SCT. CRV disease was diagnosed at a median of 343 days after SCT (range: 1-4361), with 33 cases (24%) occurring before day +100. During the infectious episode 31 of 83 allo-SCT recipients (37%) had active GVHD and 39 (47%) were under

prednisone (PDN) > 15 mg/kg. Most of the patients (84%) had symptoms compatible with an upper respiratory tract infection (URTI), with 15 of them (11%) progressing to a LRTI, while 7 (5%) had a LRTI only. Hospital admission was required in 46 episodes (33%) with a median duration of hospitalization of 11 days (range: 1–30), 17% required supplemental oxygen, 6% were transferred to the intensive care unit and 4% required mechanical ventilation. The most commonly identified pathogens over time are shown in Figure 1. Twenty-four cases (17%) had concomitant bacterial or fungal infections. Influenza A virus was the most frequent CRV detected (49 episodes, 36%) followed by human respiratory syncytial virus (37 episodes (27%) and human parainfluenza virus type 3 (18 episodes, 13%). During the 2009 Flu Pandemic, only 2 of the 13 CRV infections diagnosed in SCT recipients (15%) were associated to Influenza A virus H1N1. Antiviral treatment was started in 62 episodes (45%), antibiotics in 56% and combined therapy in 25% during a median of 7 days (range: 4–21). The RV resolved in 129 cases (94%) at a median of 12 days (2–72) from onset, with CRV being considered the leading cause of death in only 3 patients (3% of all cases and 3/22 (14%) in those with a LRTI). Predictors of severe CRV infection (including ICU admission, need for supplemental oxygen, need for mechanical ventilation requirement or death) in multivariate analysis were lymphocyte count < 200 cells/μl (HR: 4.2, 95% CI: 4–26,  $P=0.01$ ) and co-infection with other pathogens (HR: 4.8, CI95%: 1.5–16,  $P=0.00$ ). No specific CRV nor period post-SCT of the infection influenced the risk of severe infection. Our results confirm that CRV infections are a frequent cause of morbidity after SCT with a high need for hospital-based care. Temporal changes in the principal circulating CRVs has been identified during the 7-year study period, with Influenza A virus being the most common. Profound lymphocytopenia and presence of co-pathogens are associated with infection severity.

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FLUA: Influenza A virus; FLUB: Influenza B virus; PI1: Parainfluenza virus 1; PI3: Parainfluenza virus 3; RSV: Respiratory syncytial virus; MPV: Metapneumovirus; ADV: Adenoviruses

**Disclosure of conflict of interest:** None.

#### P247

##### Cost effective outcome comparison of hematopoietic stem cell transplant performed in HEPA and non-HEPA filter rooms: experience from a single center in India

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The 127 consecutive HSCT performed from 2012 to 2016 are being analysed retrospectively. Out of them 60 (47%) performed in HEPA filter room and 67 (53%) in non-HEPA filter room, criterion was purely financial to make this decision. 96/127 (76%) were Allogeneic and 31/127 (24%) were Autologous HSCTs. Blood cultures both bacterial and fungal were taken at onset of fever and with every change of antibiotics till patient became afebrile. Chest x-ray and if required HRCT chest was done for all patients who had respiratory complaints. We did not use antibacterial prophylaxis; however, antifungal prophylaxis was administered along with conditioning; and at the onset of fever systemic

antibiotics were started. Antifungal agents were added if fever persisted for 3 days pre empatically. Extremely well trained nurses were looking after both the groups. All treatment protocols, antibiotic/antifungal policies were same in both the groups. Median time for neutrophil engraftment was 12 days in HEPA filter room and 13 days in non-HEPA filter room. Total 4/127 (3 %) patients did not engraft till 30 days. Out of them 02/60 (3 %) were in HEPA filter room and 2 /67 (2.9 %) in non HEPA filter room. Blood cultures were positive in total 20/127 (16%) patients, 17 were positive for bacterial and 3 for fungal organisms. In HEPA filter HSCT 11/60 (18%) were positive and in non-HEPA filter HSCT were 09/76 (13%) positive. Total 34/127 (27%) patients developed pneumonia, out of them 14/60 (23%) were in HEPA filter and 20/67 (30%) HSCT were in non-HEPA filter room. Statistically not significant. No central venous access catheter issues or infections were documented in any groups Gr 2–4 aGVHD : HEPA rooms 11/60 (18.3%), Non HEPA rooms 14/67 (20.8%) : was not statistically significant The 30-day mortality was 25/127 (20%), 07/60 (12%) patients were from HEPA filter rooms and 18/67 (27%) were from non-HEPA filter rooms. Cost : Average cost of Allogeneic HSCT in HEPA Room : USD 22 000. Average cost of Allogeneic HSCT in Non HEPA Room: USD 13 700. Average cost of Auto HSCT in HEPA Room: USD 12 000. Average cost of Auto HSCT in Non HEPA Room : USD 8500. Incidence of Blood culture positivity & Incidence of Pneumonia was not different. These are two very important issues in outcome of HSCT. aGVHD incidence did not depend on the room type. These are significant findings from this study. Results were slightly better in HEPA filter rooms compare to non-HEPA filter rooms, which was statistically insignificant. Our study had few confounding factors hence we could not be concluded that HEPA-filtered rooms are not necessary. Nevertheless, our experience suggests that availability of dedicated HEPA units with special air-handling equipment should not be considered a critical and essential precondition for providing allogeneic HSCT to patients even in developing world with financial constraints. These would otherwise succumb to potentially curable hematological illnesses with background of financial constraints and wait list of HEPA filter rooms. Early HSCT in a CLEAN patient in Non HEPA rooms is extremely cost effective with comparable outcomes. Nursing Care, Experience of the team, Experience in HSCT program & well established protocols are MORE important in outcome of HSCTs.

**Disclosure of conflict of interest:** None.

#### P248

##### Cytomegalovirus pneumonitis complicated by a central peribronchial pattern of organising pneumonia

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We present five cases of cytomegalovirus (CMV) pneumonitis occurring in patients after recent allogeneic stem cell transplantation (AlloHSCT). These cases were complicated by an organising pneumonia (during the recovery period) with a predominantly central peribronchial pattern. All patients presented with evidence of active CMV pneumonitis which was treated successfully with anti-viral therapy but was followed by persistent severe dyspnoea, cough and hypoxia. High resolution computed tomography (HRCT) imaging showed widespread central peribronchial consolidation with traction bronchiectasis. In most cases there was a marked clinical and physiological improvement after treatment with systemic corticosteroids. However, in all patients the lung function remained abnormal and in some cases imaging revealed a fibrosing lung disease. These cases represent a previously undescribed central peribronchial pattern of organising pneumonia complicating CMV pneumonitis that can result in chronic lung damage.

**Disclosure of conflict of interest:** None.

**P249**

**Cytomegalovirus reactivation in pediatric acute leukemia after stem cell transplantation has an effect on relapse and survival in AML but not in B-precursor ALL**

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Several studies have indicated better survival after stem cell transplantation (SCT) for acute leukemias, especially acute myeloid leukemia (AML), in case of cytomegalovirus (CMV) reactivation. Here, we investigated if CMV reactivation had an impact on survival after SCT for AML or acute lymphoid leukemia (ALL) in children. 177 pediatric allogeneic stem cell transplant recipients from our institution who received myeloablative conditioning were included. Transplant indications included AML, T-ALL and B-precursor ALL. CMV reactivation was correlated with relapse, mortality as well as acute graft-versus-host disease (GVHD) and was analyzed by Fisher's Exact test or  $\chi^2$ -test (if  $n > 100$ ). From the 177 patients included, 42 were transplanted for AML (24%), 22 for T-ALL (12%), and 113 for B-precursor ALL (64%). Mortality and relapse rates (27–37% and 18–26%, respectively), CMV reactivation rates (21–36%) as well as numbers of negative CMV serology status (19–32%) of donor and recipient were comparable between different acute leukemias. When patients were analyzed altogether, CMV reactivation had no effect on relapse rates or mortality. However, a tendency towards fewer relapses after CMV reactivation was observed in AML patients (no relapse (0%) with CMV reactivation vs 11 relapse cases (33%) without CMV reactivation;  $P=0.083$ ). In those 128 leukemia patients capable of reactivating CMV (that is, donor or recipient CMV seropositive prior to SCT), CMV reactivation had a protective effect on relapse rates in AML (no relapse (0%) with CMV reactivation vs 11 relapse cases (44%) without CMV reactivation;  $P=0.017$ ). A similar tendency could be seen in T-ALL whereas no effect in patients with B-precursor ALL was documented. Numbers of acute GVHD cases grade  $>1$  between AML and T-ALL with or without CMV reactivation were similar. Different effects of CMV on relapse rates and mortality in AML vs B-precursor ALL were noticed in 79 patients who were either not capable of CMV reactivation or who did reactivate CMV post SCT. In 17 AML patients, there were no relapses (0%) and 2 deaths (12%) in contrast to 17

relapse cases (27%) and 24 deaths (39%) in 62 children with B-precursor ALL ( $P=0.017$  and  $P=0.044$ , resp.). Latently CMV infected AML patients without documented CMV reactivation after SCT have a significant worse prognosis compared with all other AML patients. This is also likely to be the case in patients with T-ALL, however, patient numbers in our cohort were too few. The protective effect of CMV reactivation in AML and possibly T-ALL does not appear to be GVH-related since the rate of relevant acute GVHD cases was comparable. CMV reactivation after SCT for B-precursor ALL lacks significance. **Disclosure of conflict of interest:** None.

**P250**

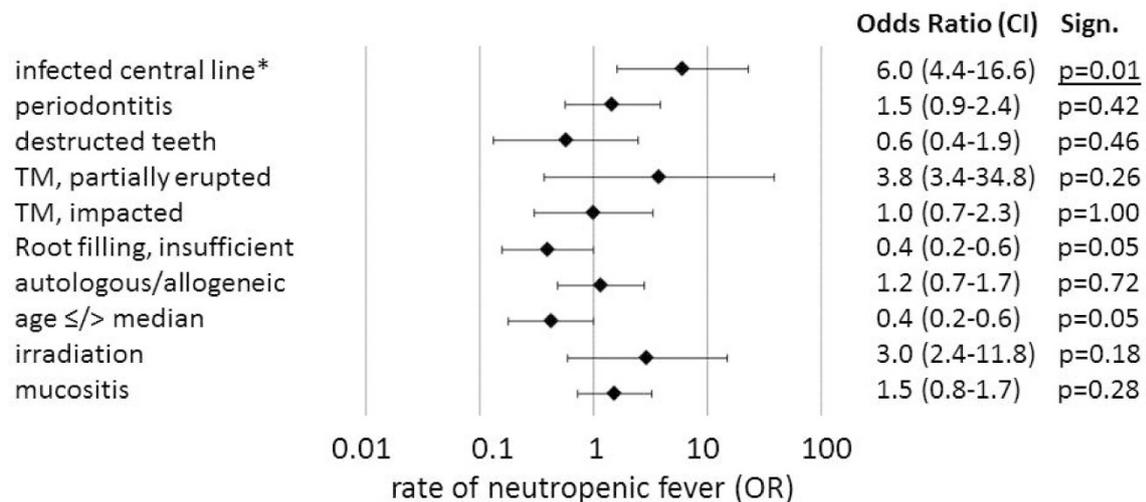
**Dental status does not predict infection during stem cell transplantation: a single-center survey**

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Infections are among the most frequent and relevant complications of hematopoietic stem cell transplantation (HSCT). Little is known about the role of dental foci for the prevalence of infections in HSCT. Dental status was prospectively evaluated in all patients at our center before undergoing HSCT. A total of 132 different patients before undergoing 163 HSCT (83 allogeneic and 80 autologous), with a median age of 52 years (range: 4–70 years) were evaluated. For evaluation a panoramic X-ray evaluation was performed. Dental findings included the status of third molars and root fillings as well as caries, periodontitis, destructed teeth and apical bone loss. As non-dental parameters we used age, sex, type and status of central venous line, mucositis, and type of transplantation. These were correlated with neutropenic fever, bacteremia and pneumonia in a bivariate manner before a multivariate analysis was performed. No correlation of initial dental status to neutropenic fever, bacteremia or pneumonia was found. However, bacteremia and suspected infection of central venous lines was a significant predictor of neutropenic fever. In conclusion, dental surgery should only be performed prior to HSCT if urgently required and limited to those individuals with overt infection. **Disclosure of conflict of interest:** None.

[P250]



TM= third molar

**P251****Early experience with CliniMACS prodigy CCS method in generation of virus-specific T-cells for pediatric patients with severe viral infections after hematopoietic stem cell transplantation**

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Viral reactivation especially in children is a frequent complication of allogeneic hematopoietic stem cell transplantation. Most of these episodes can effectively be controlled by an antiviral or antibody therapy; in refractory cases a novel virus-specific T-cell therapy could be a promising management option. In our pediatric cohort of 43 allogeneic transplantation during 1 year 9 patients fulfilled criteria for virus-specific T-cell therapy (5 boys, 4 girls, median age of 11 (1.5–16) years). Six patients were transplanted because of hematological malignancies and 3 for inborn errors. Donor distribution was the following: 7 matched unrelated, 1 sibling and 1 haploidentical donor. In 5 cases bone marrow, 3 cases peripheral blood and 1 case cord blood was used as a stem cell source. The underlying viral illness was CMV in 3, EBV in 2 and adenovirus in 1 case, while more than one virus was detected in 3 cases (CMV+adenovirus 2 cases, CMV+EBV 1 cases). Viral diseases necessitating a T-cell therapy were CMV pneumonitis and colitis, adenovirus enteritis and cystitis and PTLD. Patients initially received cidofovir for adenovirus, rituximab for EBV and a combination of gancyclovir and foscarnet for CMV infections. The indication for T-cell therapy was progressive viral disease in 8 of the 9 cases and uncontrollable viral load in 1 case. The procedure was performed on a median of 63 (49–113) day post transplant. Donors were 1st degree relatives in 5 cases, 2nd degree relatives in 3 cases and an unrelated person in 1 case, the best HLA match was haploidentical. The median age of the donors was 47 (33–60) years. Cells were produced by the CliniMACS Prodigy cytokine capture system (CCS) method after mononuclear leukapheresis. The system produced a median of 9.9 (6.7–25) times 10<sup>3</sup>/kg CD4+ and a median of 32.6 (16–125.1) times 10<sup>3</sup>/kg CD8+ interferon producing cells while the non-interferon producing cells were far below GvHD limit with a median of 3.6 (1.5–12.4) times 10<sup>3</sup>/kg CD4+ and a median of 3.85 (1.4–4.5) times 10<sup>3</sup>/kg CD8+ cells. The T-cell products were administered uneventfully in all but one case. We observed a manageable cytokine storm in one patient. Glucocorticoid treatment was ongoing due to acute GvHD in 5 children; however we could manage to keep the steroid dose below 1 mg/kg in all cases. Eight patients became completely asymptomatic, while 7 also cleared the virus. We experienced decreasing viral load in all cases, the first negative viral results were achieved on a median day of 13 (13–55). Six patients are alive without viral illness or sequele, and complete viral DNA clearance in peripheral blood with a median follow up of 329 (144–580) days. One patient with CMV pneumonitis improved during the first week but deteriorated on the second week and died of respiratory insufficiency despite of mechanical ventilation. In 2 cases the viral illness improved or cleared, but the patients died of invasive aspergillosis. No cases of GvHD, rejection, organ toxicity or recurrent infection were noticed. Virus-specific T-cell therapy produced by the CliniMACS Prodigy CCS is a feasible, fast, safe and effective way to control resistant viral diseases after pediatric hematopoietic stem cell transplantation. This treatment can be implemented within a week in most cases. In order to define the appropriate place of this approach

for patients with viral reactivations more data should be collected.

**Disclosure of conflict of interest:** None.

**P252****Early removal of central venous catheter is associated with lower rates of febrile episodes and infective complications in early post transplant period after stem cell transplantation**

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Central Venous Catheter (CVC) is essential for the treatment of recipients of stem-cell transplant. It is usually placed for the administration of conditioning regimen, stem cell infusion, intravenous antibiotics, immunoglobulins, electrolyte and nutritional support and blood concentrates. This patient group is at high risk for catheter-related bloodstream infections that can result in substantial morbidity and mortality. The neutropenia secondary to the conditioning regimen determines the risk of catheter-related infections, which may serve as an entry into the blood circulation, leading to bacteremia, fungemia, and consequently to septic shock and death. The risks of infection and the spectrum of infectious syndromes differ according to the type of transplant, conditioning regimen, type of implant of stem cells and therapies used after the procedure. Gram-positive bacteria, particularly coagulase-negative Staphylococcus spp, remain the leading cause of catheter-related bloodstream infection, although an increase in Gram-negative bacteria as the causative agent has been noted. Aim of the study: to evaluate the impact of the early CVC removal on the frequency of febrile episodes and infections in our group of patients. During a 15 years period we have treated 351 patients with hematologic neoplasm with high-dose chemotherapy and stem cells transplantation. Patients were treated in sterile room conditioned with HEPA filtration. In every patient was introduced double-lumen CVC (321 subclavia, 7 jugular, and 23 femoral). 44% were febrile (10% FOU), Catheter-related infection was present in 9%, while positive culture from CVC was present in 28%. The most frequent isolated bacteria from CVC were Gram positive–Staphylococcus coagulasa negative. The catheter was removed on the day of discharge. TRM is 2.4%. From 01 January 2016 to 01 November 2016 we have transplanted additional 40 patients. To aim to decrease infection related mortality we perform strategy to remove CVC on day +2 after stem cell transplantation. The febrile episodes decreased on 25% (10/40), there were no early post-transplant mortality due to infection. Early removal of the CVC and adequate handling from the nursing staff is essential for outcome of this patient population in regard of infective complications. Efficient prevention, early diagnosis, and effective treatment of catheter related infection are essential to providing the best care to these patients and can minimized morbidity and mortality.

**Disclosure of conflict of interest:** None.

**P253****Previously published****P254****Efficacy of antibiotic therapy during autologous stem cell transplantation: can biochemical markers help us?**

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Fever in patients with agranulocytosis during autologous hematopoietic stem cell transplantation (autoHSCT) can be associated with non-infectious causes due to G-CSF, vancomycin, engraftment syndrome. In this case biochemical markers, such as presepsin (PSP), procalcitonin (PCT) and C-reactive protein (C-RP), can help in differential diagnosis of

[P254]

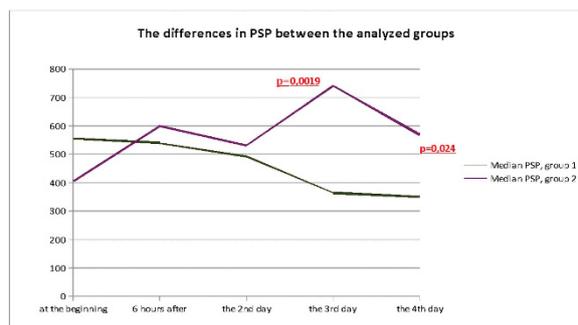
**Table 1. The dynamics of the markers (PSP, PCR, C-RP)**

PSP	Median PSP, group 1	Median PSP, group 2	PCT	Median PCT, group 1	Median PCT, group 2	C-RP	Median C-RP, group 1	Median C-RP, group 2
At the beginning	557	406	At the beginning	0.74	0.3	At the beginning	76.5	45.26
6 hours after	540.4	600.1	6 hours after	0.99	0.3	6 hours after	98.24	76.63
2nd day	492.7	532.3	2nd day	0.94	0.32	2nd day	99.69	83.3
3rd day	365.3	740.6	3rd day	0.66	0.3	3rd day	75.5	79.32
4th day	350.5	569.7	4th day	0.4	0.25	4th day	54.5	68.74

Data analysis showed that PSP decreased significantly in group 1 whereas AB therapy was effective. Presepsin is probably the only marker, testifies to the effectiveness of antibiotic therapy in patients during autologous HSCT.

fever of infectious and non-infectious genesis. PSP, PCT and C-RP were assessed on the day of admission to the hospital (DA), on D+1, on D+3, on D+7 and on the day of discharge (DD). If patients developed neutropenic fever (NF), the markers were assessed at the beginning of the fever, 6 h after, then on the second, third, fourth days after. If patients developed NF immediate empirical antibiotic therapy (AT) was implemented with meropenem. In cases of ineffective 1st line AB, 2nd line AT was added or totally changed. There were 100 patients included in the study: 41 patients with Hodgkin lymphoma, 27 with non Hodgkin's lymphoma, 32 with multiple myeloma, out of 100 patients there were 51 women and 49 men. The median age was 41 years (18–66 years). The conditioning regimens were CBV, BeEAC or HD melphalan. 69 patients developed infectious complications (IC): 2 of them had sepsis and others–NF. The median of NF development was 5.5 days. Depending on the efficiency of AT therapy patients were divided into two groups: group 1: patients that have had effective AT (they've had fast clinical response and they haven't needed to change medicine ( $n=45$ )); group 2: patients that have had ineffective 1st line AT, they haven't had response to 1st line AT and they've needed to change another AT ( $n=24$ ). There were significant differences in PSP levels on the third day after AB had been admitted: 365.3 pg/ml in group 1 and 750.6 pg/ml in group 2 ( $P=0.0019$ ). Similar differences between the analyzed groups were observed on the fourth day: 350.5 and 569.7 pg/ml, respectively ( $P=0.024$ ). PCT and C-RP didn't show any significant changes between group 1 and 2 on each day of the study (Table 1).

[P254]



**Disclosure of conflict of interest:** None.

**P255**

**Enterovirus related immune reconstitution inflammatory syndrome (IRIS) following haploidentical stem cell transplantation in an MHC class II deficient child**

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Immune reconstitution inflammatory syndrome (IRIS) has been described after HSCT in association with fungal, viral and BCG infections. We describe a case of post-HSCT IRIS associated with enterovirus infection. **Case:** A girl with MHC II deficiency (RFXP2c.362 mutation) underwent Treosulfan/Fludarabine/Thiotepa/ATG conditioned TCRαβCD3+ depleted haploidentical HSCT at 1.8 years of age. Pre-transplant work up did not reveal any viral or fungal infections except norovirus in stool. Cyclosporine (CsA) was given as GvHD prophylaxis. Neutrophil and platelet engraftment occurred on D+15 and D+9, respectively. On D+6, her stool was tested positive for enterovirus (Taqman PCR), however; she was asymptomatic. The child started having fevers and irritability from D+21 which persisted despite the use of antimicrobials. No evidence of fungal or bacterial infection was found. Enterovirus PCR in blood was found positive on D+31 (cycle threshold value, Ct 32.4) and further typing showed it to be Echovirus 13. At this time, symptoms progressed with diarrhoea, developmental regression and signs of radiculopathy. MRI (brain and spine) was normal and CSF showed pleocytosis (815 WBC/mcL–100% lymphocytes, protein 4.23 g/L) with positive enterovirus PCR (Ct 17). Subsequently, immunoglobulin prophylaxis was increased to 0.5 g/kg bi-weekly, and with supportive measures, the patient slowly recovered. Blood enterovirus PCR remained positive. With no evidence of GvHD, CsA was tapered off by day+105 and child was discharged on D+112 on a bi-weekly IVIg replacement. She presented 5 days later with signs of raised intracranial pressure. MRI showed hydrocephalus, and VP shunt was placed and broad spectrum antibiotics administered. CSF showed WBC < 1/mcL, protein 0.23 g/L and enterovirus positive. Methylprednisolone 2 mg/kg/day was started suspecting IRIS. In subsequent CSF testing 3 days later, enterovirus was negative. Enterovirus PCR remained positive in blood during this period. Patient's clinical deterioration correlated with a rise in CD4/CD8 counts and C reactive protein with clearance of enterovirus from CSF, blood

and stool (Figure 1). Subsequently, the child showed gradual but marked improvement and discharged home. **Discussion:** the clinical features of index case fits into criteria for IRIS<sup>1</sup>. Markedly raised CRP suggests high IL-6 levels without any bacterial or fungal pathogens being isolated. In addition, IRIS occurs at the site of prior active infection (brain in index case) and viral clearance and clinical recovery demonstrated with the continuation of steroids. The incidence of enterovirus infection in HSCT recipients is around 10%<sup>2</sup>. IRIS, in this case, had a temporal correlation with discontinuation of CsA, and it has been shown that discontinuation of immunosuppression is associated with higher risk of IRIS. A high index of suspicion for IRIS is necessary during immune recovery post-HSCT especially when immunosuppression is being tapered in a patient with pre-existing infection. Aggressive antiviral treatment (when available) and judicious immunosuppression are the keys to managing IRIS complications.

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[P255]

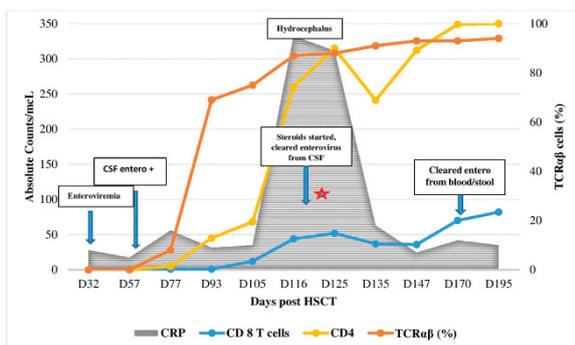


Figure 1: Immune reconstitution, clinical deterioration and recovery with clearance of enterovirus post-transplant.

**Disclosure of conflict of interest:** None.

#### P256

##### Epidemiology and risk factor assessment of PTLD in a pediatric stem cell transplantation unit: a single centre experience

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Posttransplantation lymphoproliferative disease (PTLD) is a significant cause of morbidity and mortality in allogeneic stem cell transplant patients. Identifying high risk patients, routine PCR screening, early diagnosis and therapy are crucial for successful management. Patients and Methods Primary objectives of this study were to describe epidemiology of EBV associated PTLT and to assess risk factors in our paediatric cohort. Additionally, role of immunoglobulin (Ig) levels as a possible diagnostic/prognostic marker was analyzed. Between 1 January 1 2011 and 30 June 2016, 140 allogeneic transplantations were performed in 118 pediatric patients (82 boys and 36 girls) at our center. Median age was 7.68 years (0.03–18). Underlying diseases were hematological malignancies (68%), nonmalignant hematological conditions (13%), immunodeficiencies (11%) and others (8%). Stem cell source was bone marrow (62%), peripheral blood (19%) and cord blood (19%). Donors were unrelated (75%), sibling (18%),

haploidentical (4%) or other matched family donors (3%). Routine EBV PCR screening and Ig level detection were performed weekly. Rituximab prophylaxis was given only in nine cases. Results EBV DNAemia was found in 16/118 patients (13.6%), while PTLT was diagnosed in 11/118 patients (9.3%). All PTLT cases were related to EBV infection, median of highest viral load was 11 790 copies/ml (688–6 670 000). Diagnosis was confirmed by biopsy in 6/11 cases, further five fulfilled criteria of probable PTLT (positive PCR with appropriate clinical symptoms). PTLTs occurred at a median of day +48 (19–85) after transplantation. All patients received rituximab treatment along with a reduction of immunosuppressive therapy. Four patients died of PTLT (mortality 36%), all confirmed by autopsy. A higher incidence of male gender (10/11; 90.9% vs 67.3%), bone marrow graft (10/11; 90.9% vs 59.8%), hematological malignancy (10/11; 90.9% vs 67.3%) and second transplantation (5/11; 45.5% vs 14%) could be detected among PTLT patients when compared to the non-PTLT group. Elevated IgG, IgA or IgM levels were observed in 13/118 patients. Nine out of 13 had positive EBV PCR testing, eight of them developed PTLT. Five of the PTLT patients had monoclonal or biconal immunoglobulin elevation, two of them died. In 3 cases, elevated Ig level preceded the positive EBV PCR results by at least 1 week.

**Conclusion:** At our centre incidence and mortality of PTLT was similar to published data. We observed a tendency that a higher representation of male gender, hematological malignancy, bone marrow graft and second transplantation could be confirmed in the PTLT group however due to small number of patients, a correlation and statistical significance could not be calculated. Elevation of immunoglobulin levels do not seem to be specific for PTLT but in selected cases it could predict EBV disease earlier than PCR testing.

**Disclosure of conflict of interest:** None.

#### P257

##### Infections in patients treated with autologous peripheral hematopoietic stem-cell transplantation

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Autologous peripheral hematopoietic stem-cell transplantation is a procedure of a stem cell rescue with patients' own previously collected hematopoietic stem cells, after myelotoxic therapy. The purpose of stem cell reinfusion is to ensure adequate recovery of hematopoiesis, shorten the period of profound neutropenia and to reduce the risk of infections. The transplantation itself carries a moderate risk for infection but some patients have higher risk due to the nature of underlying disease, earlier treatment and in case of severe mucositis. For these reasons, all treated patients are in isolated clean rooms and receive ciprofloxacin, fluconazole and acyclovir prophylaxis. In the 3.5-year period, 177 autologous transplantations were performed. The patients were 20–72 years old, with median of 55.18 years. Of all transplanted patients, 106 or 59.88% had multiple myeloma, 66 or 37.3% had lymphoma and 5 or 2.82% had acute myeloid leukemia. All of the patients received pegfilgrastim 6 mg on the first or the second post-transplant day. Febrile neutropenia (Ne < 0.5 × 10<sup>9</sup>/L) was reported if patient's temperature was above 38.3 °C in one measurement or above 38 °C in two consecutive measurements. These patients were treated empirically with piperacillin/tazobactam 4.5 g four times a day with the addition of vancomycin in the case of severe mucositis or pulmonary infiltrates. In all cases blood and urine cultures were performed, as well as testing for seasonal flu. Time to

neutrophil recovery ( $Ne > 0.5 \times 10^9/L$ ) was 7–20 days, with a median of 10 days, and average of 10.25 days. Febrile neutropenia was reported in 76 patients (42.94%) and in 39 (51.31%) patient's samples pathogen was isolated. Gram negative bacteria caused sepsis in 54.29% of patients. We had to change empirical therapy according to antibiogram in 37.2% patients. In 1 month follow-up period, there were two (1.12%) infection related deaths. Our data on incidence of infections is consistent with literature data but large number of papers show satisfactory results of safety of patients discharged from hospital immediately after the autologous stem cell transplantation and who were treated at home during the phase of profound neutropenia. There is still an ongoing debate whether it is possible to conduct this procedure in such manner in our health system.

**Disclosure of conflict of interest:** None.

**P258**

**Fluconazole was equal to mold-active drugs in preventing early invasive fungal disease after allogeneic stem cell transplantation regardless of transplantation type**

*Y Sun, J Hu, H Huang, J Chen, J-Y Li and X-J Huang*

There are still controversies that whether mold-active drugs is better than fluconazole in preventing invasive fungal disease

(IFD) after allogeneic stem cell transplantation (HSCT). We hypothesised that the optimal prophylaxis might be different in patients with different risk profile, such as in different time period after HSCT or received alternative donor transplantation. In the prospective China Assessment of Antifungal Therapy in Haematological Disease (CAESAR) study database, 661 out of 1401 patients received primary antifungal prophylaxis were analyzed. The IFD incidence of different time period after transplantation (early, late and very late) and survival were compared among different drug groups. In patients with fluconazole, itraconazole, voriconazole or micafungin prophylaxis, the overall incidence of IFD after transplantation were 7.2%, 12.6%, 1.4% and 5.2%, respectively ( $P = 0.0379$ ). However, there is no difference in early IFD ( $< 40$  days post HSCT) among 4 groups of patients. The risk factors associated with occurrence of IFD were neutropenia duration  $> 14$  days ( $P < 0.01$ , OR 3.73 (1.66–8.36)), adult ( $P = 0.02$ , OR 3.37 (1.23–9.18)) and alternative donor (unrelated donor or haploidentical donor) transplantation ( $P = 0.01$ , OR 5.88 (1.48–23.32)). In the sub-group analysis with only alternative donor (unrelated donor and haploidentical donor), it also demonstrated that fluconazole is equal to other mold-active drugs in preventing early IFD. Patients received fluconazole prophylaxis has even better overall survival. The overall survival in patients with fluconazole, itraconazole, voriconazole or micafungin prophylaxis were 88.3%, 83.5%, 78.9% and 72.4%, respectively ( $P = 0.0047$ ). Our current

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**Table 1**

Pts tot 4 (3.7%)	Disease	HSCT	HBV Recipient	HBV Donor	Rituximab	Duratio on Prophylaxis (Months)	R- HBV DNA/ HBsAg	Months to react.afterstopping LVM
Pts 1	LNH	MUD	HBcAb+ HBsAb+ HBeAb+ HBsAg- HBV DNA-	HBsAg- HBcAb-	Yes	52	42 UI/mL HBsAg-	4
Pts 2	LLC	NO	HBcAb+ HBsAb+ HBeAb+ HBsAg- HBV DNA-	/	Yes	54	69534 UI/mL HBsAg+	3
Pts 3	LAM	HLA-ID	HBcAb+ HBsAb+ HBeAb- HBsAg-	HBcAb+ HBsAb+ HBsAg-	No	50	<20 UI/mL HBsAg-	6
			HBV DNA-					
Pts 4	LLC	HLA-ID	HBcAb+ HBsAb+ HBeAb- HBsAg- HBV DNA-	HBcAb- HBsAg- HBsAb+	Yes	38	<20 UI/mL HBsAg-	3

Prolonged LMV prophylaxis was associated with HBV reactivation in 7.4% of haematological patients. In 3.7% HBV reactivation was documented months after lamivudine suspension. A careful monitoring of possible HBV-DNA reappearing is needed in haematological patients also at the suspension of treatment. The use of more potent antiviral drugs should be considered in haematological patient at higher risk of HBV reactivation.

**Disclosure of conflict of interest:** None

study suggests that fluconazole is equal to mold-active drugs to prevent early IFD in HSCT patients, even in high-risk patients received transplantation from alternative donors. However, further prospective randomized study was warranted to confirm this conclusion.

**Disclosure of conflict of interest:** None.

**P259**

**HBV reactivation despite antiviral prophylaxis in haematological patients: results after 2 years of monitoring**

C Cerva, A Ricciardi<sup>1</sup>, G Maffongelli<sup>1</sup>, G Malerba<sup>1</sup>, V Malagnino<sup>1</sup>, G De Angelis<sup>2</sup>, R Cerretti<sup>2</sup>, L Cudillo<sup>2</sup>, R Salpini<sup>3</sup>, A Battisti<sup>3</sup>, L Colagrossi<sup>3</sup>, M Pollicita<sup>4</sup>, V Svicher<sup>3</sup>, CF Perno<sup>5</sup>, W Arcese<sup>2</sup>, C Sarrecchia<sup>6</sup>, M Andreoni<sup>6</sup> and L Sarmati<sup>6</sup>

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HBV reactivation is a serious event in haematological setting. Duration of prophylaxis and follow up still remain debated themes and viral reactivation can occur after withdrawal of antiviral drugs despite recommended prophylaxis treatment. Study population is composed by 107 haematological patients, seropositive for HBV, quarterly evaluated in a follow up program by December 2014: 42 patients (39.2%) underwent haematopoietic stem cells transplantation (HSCT), 4 (9.5%) received autologous HSCT and 38 (90.5%) allogeneic HSCT. Sixty-five out of 107 patients (60.7%) were affected by different haematological diseases: 36 by lymphoma, 11 by multiple myeloma, 7 by chronic lymphocytic leukaemia and 11 by others diseases including mastocytosis, amyloidosis and essential thrombocythemia. HBV reactivation prophylaxis

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**Conditioning regimen:**

- VP-16 and Total Body Irradiation (TBI)
- TBI and Fludarabine
- Fludarabine/Busulfan/Thiotepa
- Busulfan & Fludarabine x 4

**Transfusional support:**

	Platelet pools	Red cell concentrates
Patient 1	100	60
Patient 2	10	20
Patient 3	37	35
Patient 4	24	18

**Evolution of Poliovirus in serum:**

BKV serum at the diagnosis BKV in serum at the time of resolution

Patient 1: 14.864.927 copies/ml. 1.102.147 copies/ml.

Patient 2: 387 copies/ml. Negative

Patient 3: 568 copies/ml 12 copies/ml.

Patient 4: Negative

**Copies of BK virus in urine at the diagnosis:**

— Patient 1: 1000.7 copies/ml.

— Patient 2: 29.610.645 copies/ml.

— Patient 3: 3.972.230 copies/ml.

— Patient 4: 1.952.560 copies/ml.

prescribed was entecavir for 4 HBsAg+ inactive carrier patients and prolonged lamivudine (LMV) course for 103 (96%) patients. In 6 patients (5.6%) LMV prophylaxis was withdrawn 12–18 months after the end of immunosuppressive therapy. Eight out of 107 patients (7.4%) experienced HBV reactivation: 4 of them during LMV treatment and then they were switched to entecavir or tenofovir therapy, 4 patients reactivate HBV after LMV interruption (3.7%). In these patients reactivation was observed after an average time of 4 months (range: 3–6) after discontinuation of LMV prophylaxis. Median duration of prophylaxis was 49 months (range: 40–60) after the end of immuno-suppression. Three out of 4 patients (75%) underwent allogeneic HSCT and 3 patients (75%) received Rituximab. One out of 4 (25%) seroreverted in HBsAg positive and HBsAb negative status, with HBV-DNA > 2000 UI/mL (Table 1). Two patients out of 4 (50%) experienced HBV-DNA detection below 20 UI/mL.

**Disclosure of conflict of interest:** None.

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**Hemorrhagic cystitis: a review of our experience**

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Hemorrhagic cystitis (HC) is suspected when hematuria is observed macroscopically or in urinalysis in the early stage of post transplant (most related to chemotherapy) or post-engraftment period (associated to virus like Poliovirus).

[P260]

**HEMORRHAGIC CYSTITIS: A REVIEW OF OUR EXPERIENCE**

- **INTRODUCTION:**  
Hemorrhagic cystitis (HC) is suspected when hematuria is observed macroscopically or in urinalysis in the early stage of post transplant (most related to chemotherapy) or post-engraftment period (associated to virus like Poliovirus). The severity is measured on grades (Grade 1: microscopic hematuria to Grado 4: clots cause urinary tract obstruction).  
The treatment is based on support measures: hyperhydration, continuous bladder irrigation, instillation of topical agents and in severe cases must be performed a cystoscopy for clot evacuation. In the case of the presence of Poliovirus virus (BK virus) the use of Cidofovir had been demonstrated in vitro studies to have activity against BK virus.
- **METHODS:**  
We performed 42 allogenic transplants of which 10 are haploidentical from 2010 to October 2016. We realized a retrospective case study to analyses the experienced in the management of HC.
- **RESULTS:**  
Of a total of 42 allogenic transplants realized, developed a HC: 1 patient received an identical HLA transplant and the 4 patients remaining haploidentical allotransplant. All cases were male, with an age range of 18-42 years.  
The status of the disease was: 3 were in complete remission and 1 had visible disease.  
3 of the 4 patients received cyclophosphamide as immunosuppressive therapy and all patients received cyclosporine and mofetil micofenolate also.  
The onset of the symptomatology was between day 9 to day 82 post transplant and the range of duration was from 14 to 45 days.  
Conditioning regimen:  
--- VP-16 and Total Body Irradiation (TBI)  
--- TBI and Fludarabine  
--- Fludarabine/Busulfan/Thiotepa.  
--- Busulfan & Fludarabine x 4.

**Transfusional support:**

	Platelets pools	Red Cells concentrates
Patient 1	100	60
Patient 2	10	20
Patient 3	37	35
Patient 4	24	18

**Evolution of Poliovirus in serum:**

	BKV serum at the diagnosis	BKV in serum at the time of resolution
Patient 1:	14.864.927 copies/ml.	1.102.147 copies/ml.
Patient 2:	387 copies/ml.	Negative
Patient 3:	568 copies/ml	12 copies/ml.
Patient 4:	Negative	

The severity is measured on grades (Grade 1: microscopic hematuria to Grado 4: clots cause urinary tract obstruction). The treatment is based on support measures: hyperhydration, continuous bladder irrigation, instillation of topical agents and in severe cases must be performed a cystoscopy for clot evacuation. In the case of the presence of Poliomavirus virus (BK virus) the use of Cidofovir had been demonstrated *in vitro* studies to have activity against BK virus. We performed 42 allogenic transplants of which 10 are haploidentical from 2010 to October 2016. We realized a retrospective case study to analyses the experienced in the management of HC. **Results:** Of a total of 42 allogenic transplants realized, developed a HC: 1 patient received an identical HLA transplant and the 4 patients remaining haploidentical allotransplant. All cases were male, with an age range of 18–42 years. The status of the disease was: 3 were in complete remission and 1 had visible disease. 3 of the 4 patients received cyclophosphamide as immunosuppressive therapy and all patients received cyclosporine and mofetil micofenolate also. The onset of the symptomatology was between day 9 and day 82 post transplant and the range of duration was from 14 to 45 days. The four patients precised continuous bladder irrigation but because of the poor response they received instillation of hyaluronic acid (4 doses). Two patients required the use of Cidofovir (3 doses). One of the four patients required urinary tract catheterization because of hydronephrosis and renal impairment. In our review we confirmed that this entity is more frequent in the haploidentical transplant and BKV is the most prevalent cause in the late HC. - The three patients received 3 doses of Cidofovir (1mg/kg) without probenecid and had a good response. - Three patients present acute renal failure associated to HC. The four patients needed bladder instillations with saline but they had poor response and received at least 4 doses of hyaluronic acid.

**Disclosure of conflict of interest:** None.

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#### Herpes simplex virus (HSV) infection in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT)

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HSV infection in allo-HSCT setting is mostly reactivation of latent virus. HSV disease commonly presents as mucocutaneous lesions of the oral cavity. However some patients develop serious fatal visceral dissemination. Prophylactic use of acyclovir has markedly reduced the incidence of HSV disease during the period of neutropenia after allo-HSCT. In this study, our aim is to demonstrate the incidence, clinical outcome and risk factors for HSV disease in adult allo-HSCT. Between 2015 and 2016, 89 patients who underwent allo-HSCT in our center were included to the study. All HSCT candidates and donors were tested for HSV-1/2 immunoglobulin G (IgG) antibodies prior to transplantation. All patients received acyclovir prophylaxis (related transplants 400mg TID, unrelated transplants 800mg TID) during conditioning and after allo-HSCT up to 3 months. Chlorhexidine oral solution as well as bioadherent oral protective gels was used for oral hygiene. All patients were followed for symptoms of reactivation. HSV1/2 IgG seropositivity was detected in 66 recipients (74%) and 48 donors (54%). The distribution of HSV status was as follows: Recipient and donor seropositive in 34 (38%), recipient and donor seronegative in 9 (10%), recipient seropositive and donor seronegative in 32 (36%), recipient seronegative and donor seropositive in 14 (16%) transplants. The median age of the patients was 41 (range: 18–67), 48 patients were male (54%) and 79 (89%) had malign disease. The stem cell source was peripheral blood in 73 (82%) patients and 48 (54%) received grafts from related donors. Sixty four patients (72%) received myeloablative conditioning regimen. The most common graft-vs-host disease (GVHD) prophylaxis administered was cyclosporine (CSA) and methotrexate (Mtx) in 60 patients (67%). Acute graft vs host disease was detected in 29 patients (33%). Four patients from seropositive 66 patients (6%) had HSV reactivation, the patient characteristics are given in the table. All patients had HSV reactivation within 1 month of allo-HSCT except one patient had symptoms at sixth month posttransplant when he suffered from oral GVHD. All patients

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**Table. Patients with HSV reactivation (AML: Acute Myeloid Leukemia, ATG: Anti-thymosin globulin, Bu: Busulphan, Cy: Cyclophosphamide, Flu: Fludarabine, F: Female, M: Male, PB: Peripheral Blood)**

Patient/ Sex/ Age	Diagnosis	Donor type/Stem Cell Source	Conditioning Regimen/GVHD prophylaxis	Time of HSV reactivation (posttransplant day)/symptom	Treatment/Duration	Outcome
1/F/51	AML	Unrelated/PB	Bu+Cy+ATG/ CSA+Mtx	11/herpes labialis	Valacyclovir /7days	Responded to treatment
2/M/22	AML	Related/PB	Bu+Cy/CSA+Mtx	9/herpes gingivostomatitis	Valacyclovir/30 days	Responded to treatment
3/60/M	AML	Unrelated/PB	Flu+Bu+ATG/CS A+Mtx	189/herpes labialis	Valacyclovir/7 days	Responded to treatment
4/66/F	MDS	Related/PB	Flu+Bu/CSA+Mt x	13/herpes labialis	Acyclovir/7 days	Responded to treatment

and donors were seropositive prior to allo-HSCT and responded well to antiviral treatment. The incidence of HSV reactivation in allo-HSCT was detected as 6% which is lower to previous studies. Successful primary prophylaxis and oral hygiene might reduce the incidence. All patients were responded to antiviral treatment and no visceral dissemination was detected.

**Disclosure of conflict of interest:** None.

#### P262

##### **How hematopoietic stem cell transplantation affects the acquired immunity of immunopreventable diseases**

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Patients who have received hematopoietic stem cell transplantation (HSCT) may suffer, to some extent, losses in humoral and cell immunity against antigens to which they had been previously exposed naturally (infection caused by wild microorganisms) or artificially (through vaccination). The conditioning regimen for HSCT replaces the patient's immune system and involves the loss of previous immunity. This study analyzed patients included in the vaccination program for HSCT recipients in the Salamanca Health Care Complex during the period 2010–2016. We assessed the serological status prior to HSCT for the following immunopreventable diseases (hepatitis B, hepatitis A, varicella), and the study after HSCT also included measles, rubella and parotitis, prior to their inclusion in the HSCT vaccination program. The study included 302 patients, 53.8% of which ( $n=168$ ) were men. 83.3% of the patients ( $n=260$ ) were allogeneic HSCT recipients with an average age of  $47 \pm 16$  years, and 16.7% (52) were autologous HSCT recipients with an average age of  $43 \pm 16$  years. Prior to HSCT, 40% of the patients showed immunity against hepatitis B (HBV antibodies  $>10$  UI/L), 82.6% against hepatitis A (positive for HAV IgG) and 85% against varicella (positive for varicella IgG). No statistically significant differences were observed regarding this variable Hepatitis B Anti-HBs  $>10$  UI/L, Hepatitis A, IgG positive, Varicella IgG positive, Measles IgG positive, Rubella IgG positive, Parotitis IgG positive. Table 1 compares the serological status before and after transplantation. In the pre-transplant serological study we observed that less than half of the patients are protected against hepatitis B, while over 80% of them are protected against hepatitis A and varicella. Regarding the diseases in which we know the serological status before and after transplantation (hepatitis A, hepatitis B and varicella), we observed that most patients maintain immunity. In the case of rubella, measles and parotitis we only have access to the serological status after transplantation, and we observed that parotitis is the disease with the lowest seroprotection. Therefore, vaccination would be indicated, just as in the case of hepatitis B. The clinical results support the need to adapt the vaccination schedule to the immunological status of the patients after HSCT individually.

**Disclosure of conflict of interest:** None.

#### P263

##### **Impact of cumulative steroid dose on infectious diseases after allogeneic hematopoietic cell transplantation**

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After allogeneic hematopoietic cell transplantation (HCT), high-dose steroids are used to treat transplantation-related complications such as graft-versus-host disease (GVHD). However, the use of high-dose steroids is associated with an elevated risk of

infectious diseases. Information on the association between cumulative steroid dose and infectious diseases after HCT is scarce. A total of 238 patients who underwent their first HCT in Kyoto University Hospital from 2005 to 2015 and survived at least 30 days after transplantation were included in this study. We analyzed the association between cumulative steroid dose used within 30 days after transplantation and the occurrence of infectious diseases, including invasive fungal infection (possible/probable/proven cases), cytomegalovirus (CMV) antigenemia, and bacteremia through 180 days after transplantation. Sixty-three patients received transplantation from a related donor, 114 received unrelated bone marrow grafts, and 61 received unrelated cord blood units. Their median age was 51 (range: 17–66) years and median day of neutrophil engraftment after transplantation was 21. Patients were categorized into 3 groups according to their cumulative steroid dose within 30 days: no steroid administration ( $n=183$ ), low-dose cumulative steroid administration under 500 mg of prednisolone in total ( $n=27$ ), and high-dose cumulative steroid administration over 500 mg of prednisolone in total ( $n=28$ ). Reasons for steroid administration were treatment for GVHD in 35 patients, engraftment syndrome in 11, and other reasons including lung complications in 9. The rate of invasive fungal infection was 6% (12 possible cases with pneumonia and 1 proven case of candida blood stream infection) and we found no apparent association between fungal infection and steroid use regardless of dose. CMV antigenemia was diagnosed in 41%, 67% and 60% of patients in the 3 groups respectively, and both low-dose and high-dose steroid groups were significantly associated with a high risk of CMV antigenemia (low-dose group, adjusted hazard ratio (aHR), 2.37,  $P=0.004$ ; high-dose group, aHR 1.84,  $P=0.01$ ). Bacteremia was diagnosed in 9.8%, 11% and 21% of patients in the 3 groups, respectively. High-dose steroid use was a risk factor for bacteremia (aHR 1.70,  $P=0.027$ ). Seven patients died from infection (fungal, 2; viral, 2; bacterial, 3). Two of three bacterial infection-related deaths occurred in the high-dose steroid group, although the number of events was too small to analyze. Our data confirmed that steroid administration is itself a risk factor for CMV antigenemia and close observation to detect CMV antigenemia is mandatory for patients using steroids regardless of its cumulative dose. High-dose cumulative steroid use is a risk factor for bacteremia. Contrary to our expectations, steroid administration showed no apparent association with invasive fungal infection in our study, perhaps because of its generally low incidence in our hospital.

**Disclosure of conflict of interest:** None.

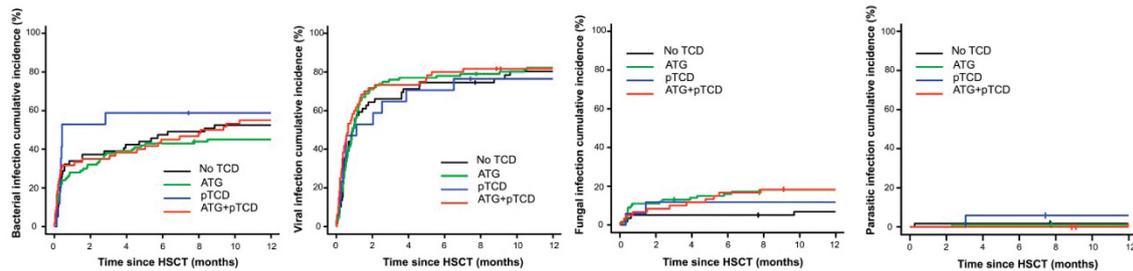
#### P264

##### **Impact of different T-cell depletion techniques on the incidence of infectious complications after allogeneic hematopoietic stem cell transplantation**

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T-cell depletion (TCD), obtained by either *in vivo* anti-thymocyte globulin (ATG) administration or *ex vivo* depletion, is a well-established strategy for Graft-versus-Host-Disease (GVHD) prevention after allogeneic hematopoietic stem cell transplantation (HSCT)1–4. However, the prolonged lymphopenia associated with TCD can result in increased incidence of disease relapse1 and infections. Although many studies investigated the impact of TCD on disease relapse 1–4, little is known about the impact of TCD strategies on the incidence of infectious complications after allogeneic HSCT. We retrospectively evaluated the incidence of infectious complications in 236 consecutive patients who underwent allogeneic HSCT at our center from September 2010 to December 2015. 100 patients received TCD grafts obtained by *in vivo* ATG administration as part of the conditioning regimen (ATG



group). 17 patients received partially TCD grafts obtained through incubation with alemtuzumab *in vitro* washed before infusion followed on day +1 by an add-back of donor T CD3+ cells5 (pTCD group). 60 patients received grafts TCD by both methods combined. 59 patients did not receive any form of TCD (No-TCD group). Cumulative incidence estimates of infectious complications were calculated and compared using the Gray test. Given the increased risk of infection associated with GvHD and its treatments, GvHD or death from other causes were defined as competitive events in the analysis. We didn't observe any significant difference in the 1-year cumulative incidence of bacterial infections in patients receiving TCD by ATG (45% (95% CI 35–54.5%)) pTCD (58.8% (95% CI 31.2–78.5%)) or both (55% (95% CI 41.4–66.7%)) compared with patients receiving No TCD (52.5% (95% CI 38.9–64.5%)). Similarly, the 1-year cumulative incidence of viral infections or reactivations was comparable in patients receiving No-TCD grafts (80.3% (95% CI 66.8–88.8%)) compared with patients receiving TCD grafts (ATG: 82.2% (95% CI 72.9–88.6%); pTCD: 76.5% (95% CI 45.7–91.2%); ATG+pTCD: 81.7% (95% CI 68.9–89.6%)). Finally, no significant impact of TCD was observed on 1-year cumulative incidence of fungal (No-TCD: 6.8% (95% CI 2.2–15.2%); ATG: 18.1% (95% CI 11.2–26.3%); pTCD: 11.8% (95% CI 1.8–31.9%); ATG+pTCD: 18.3% (95% CI 9.7–29.1%)) and parasitic (No-TCD: 1.7% (95% CI 0.1–8%); ATG: 1% (95% CI 0.1–4.9%); pTCD: 5.9% (95% CI 0.3–24.2%); ATG+pTCD: 1.7% (0.1–8.3%)) infections. Image/Graph: 1-year cumulative incidence estimates of infectious complications depending on the TCD strategy employed. The results of our retrospective analysis indicate that the cumulative incidence of bacterial, viral, fungal and parasitic infectious diseases are similar in patients receiving TCD grafts compared to those receiving No-TCD graft, suggesting a favorable toxicity profile of different TCD strategy in respect of infections. These results should be confirmed by similar analysis in large scale, prospective clinical trials assessing the potential benefits of TCD on transplantation outcomes.

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**Disclosure of conflict of interest:** None.

#### P265

##### Impact of previous invasive fungal infections on the outcome of patients undergoing allogeneic hematopoietic stem cells transplantation

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Invasive Fungal Infections (IFI) are a possible complication of allogeneic hematopoietic stem cells transplantation (HSCT)

and a prior IFI increases the patient's risk for transplant related mortality due to the possibility of reactivation of the fungal infection. This study aimed to evaluate the impact of a previous IFI history on transplant outcome. We retrospectively collected the clinical data of patients with acute myeloid leukemia (AML) considered eligible for allogeneic HSCT between 2012–2014 at the Rome Transplant Network (RTN), a JACIE accredited metropolitan transplant program established in Rome since 2006. The observation of patients was continued until 31 December 2015. The diagnosis of IFI were defined as possible, probable and proven as established by European Organization for Research and treatment of Cancer. 57 patients with AML were considered eligible for HSCT, 7 died before transplantation. 13 patients (26%) underwent transplantation from HLA-identical sibling, 13 (26%) from haploidentical family donor and 19 (38%) from matched unrelated donor, while 5 patients (10%) received unrelated cord blood cells. Twenty (35%) out of 57 eligible patients have had an IFI episode before transplant: 6 were proven, 4 probable and 10 possible; (9 (47%) pneumonia, 4 (19%) gastroenteritis, 3 (15%) sinusitis, 2 (10%) candida sepsis, 1(5%) meningitis and 1(5%) cutaneous abscess were registered). Five (25%) out of 20 patients with a previous IFI and 2 (5%) out of 37 without previous IFI did not receive HSCT (OR 5.83 95% CI 1.02–33.96, Fisher test *P*: 0.04). The majority (55%) of patients with a previous IFI waited HSCT more than 6 months from the date of eligibility in comparison with those without a previous IFI (55% vs 30%; OR 0.37, 95% CI 0.12–1.13, *P*-value 0.08 Fisher test). Overall a post transplant IFI episode was diagnosed in 13 (26%) of transplanted patient; 4 (26%) had a relapse of a past IFI vs 9 (25%) of the patients without a previous IFI who had a new episode. (OR 1.53, 95% CI 0.39–5.90, *P*-value 0.4 Yates test). A higher number of patients with IFI (10 out of 15, 66%) respect to those without a previous IFI (10 out of 35, 30%) died in a median time of 160 days (range: 22–480 ) after HSCT. Furthermore, those who had a previous IFI had a lower median survival (317 days (range: 22–1095)) compared to patients without a previous IFI (530 days period (range: 20–1490)) (Student's *t*-test *P*: 0.014). A previous IFI episode in the pre transplant period slows and limits the accessibility to HSCT, and is significantly associated with an increased mortality.

**Disclosure of conflict of interest:** None.

#### P266

##### Previously published

#### P267

##### Incidence and risk factors of invasive aspergillosis infection in patients undergoing haploidentical stem cell transplantation after prophylaxis with micafungin and oral triazole

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	Patient 1	Patient 2	Patient 3
<b>EARLY PERIOD</b>			
<b>Sex/Age</b>	F/4 yo	F/4 yo	M/70 yo
<b>Diagnosis</b>	Aplastic anemia	AML RC1	AML - Sequential HSCT
<b>Presentation</b>	Day 50, primary graft failure	Day 14, pre-engraftment	Day 17, pre-engraftment
<b>Prophylaxis</b>	Micafungin	Micafungin	Micafungin
<b>Site of infection</b>	Pneumonia (prob IAI)	Pneumonia (prob IAI)	Pneumonia + skin (definite, <i>A. niger</i> )
<b>Result</b>	Died (ARDS)	Alive, engrafted, no GVHD (2 m follow up)	Died, aGVHD grade I.
<b>LATE PERIOD</b>			
<b>Sex/Age</b>	F/9 yo	M/29 yo	M/70 yo
<b>Diagnosis</b>	Common variable immunodeficiency	NHL-T, RC3	MDS, MRD negative.
<b>Risk factors</b>	aGVHD grade IV- day 21 and grade III- 6 <sup>th</sup> m	Previously IAI. aGVHD grade II- day 28. cGVHD grade III - 6 <sup>th</sup> m. CMV disease- 7 <sup>th</sup> m.	aGVHD grade I- day 28. Autoimmune encephalitis- 6 <sup>th</sup> m, chronic steroid use. Hepatotoxicity induced by posaconazole.
<b>Presentation</b>	7 <sup>th</sup> month, probable IAI	8 <sup>th</sup> month, probable IAI	10 <sup>th</sup> , month, probable IAI
<b>Prophylaxis</b>	Micafungin	Posaconazole	Fluconazole
<b>Site of infection</b>	Pneumonia (probable IAI)	Pneumonia + SNC (probable IAI)	Pneumonia + SNC (probable IAI)
<b>Result</b>	Died (ARDS)	Died.	Died.

Delayed immune reconstitution has been described for haploidentical hematopoietic stem cell transplantation (HSCT) compared to conventional HSCT, nevertheless the incidence of invasive aspergillus infections (IAI) in haploidentical SCT and the efficacy of primary prophylaxis are not well defined. Our objective is to describe the incidence, risk factors and mortality of IAI in our patients, using as prophylaxis micafungin during the conditioning and neutropenia period, switched to posaconazole or voriconazole when oral intake is feasible. We retrospectively analyzed 40 consecutive patients from 2014 to 2016 who received haploidentical grafts: unmanipulated for 20 adults, TCRab depleted in 6 children and CD45RA depleted in 14 children. The stem cell source was peripheral blood in all cases. Adults (22–70 yo) were treated for AML/MDS ( $n=8$ ), ALL ( $n=2$ ) and Lymphoma ( $n=10$ ). Children (6 mo–15 yo) were treated for AML ( $n=6$ ), ALL ( $n=9$ ), Aplastic anemia ( $n=2$ ) and Immunodeficiencies ( $n=3$ ). Conditioning regimen was Bu-Flu-Cy ( $n=18$ , adults), Thio-Bu-Flu ( $n=2$ , adults), Flu-Mel-Thio for all pediatric patients; ATG was used in 6 children and TLI in 14 children. Median follow up was 12 months (1–30) for adults and 7 months (1–28) for children. We used EORTC criteria for IAI and analyzed probable or definite as cases. There were 6 events of IAI, with a bimodal presentation: 3 events (7.5%) during neutropenia period and 3 (7.5%) after 6 months of HSCT (Figure 1). Five of them were probable and one definite (*Aspergillus niger*). Site of infection was mainly

pulmonary; CNS was suspected in two adult patients and skin was proven in one adult patient. All 3 patients at the late period had chronic GVHD at diagnosis. One patient had primary graft failure. Severe CMV disease (hepatitis and colitis) was present in one adult. Mortality related to IAI was high (5/6), patients died at a median of 35 days. Figure 1. IAI patients characteristics. The global incidence of IAI in haploidentical HSCT is similar to conventional HSCT. Primary prophylaxis with micafungin switching to oral triazole is successful (92.5%) during the early period. Late cases (7.5%) had clearly known risk factors (chronic GVHD, steroids and CMV), and primary prophylaxis had been modified due to toxicity or interactions. IAI mortality in our patients is very high (84%) despite effort in prophylaxis, diagnosis and treatment.

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**Disclosure of conflict of interest:** None.

**P268**

**Intractable abdominal pain as first clinical symptom of VZV reactivation in patients after allogeneic stem cell transplantation: case report and review of the literature**

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Visceral intractable abdominal pain prior to skin lesions from herpes zoster can be misdiagnosed as GVHD post stem cell transplantation which may lead to initial increase in immunosuppression and hence high mortality if we don't suspect. Case report and literature review through Pubmed Results: 13-year-old male with relapsed ALL post MUD PBSCT (10/10) transplant in following Cy TBI ATG conditioning presented at Day +117 with intractable diffuse abdominal pain with constipation. No history of nausea, vomiting or skin rash. On physical examination his abdomen was soft, diffuse tenderness but no rigidity, muscle guarding and rebound tenderness. Laboratory tests including liver function test, amylase, lipase were normal. USG abdomen and MRI abdomen showed no abnormalities, except for presence of fecolith. During the stay his pain worsened needing morphine infusion, PCA and later ketamine. He had previous history of acute GUT GVHD controlled on Budesonide and Cyclosporine which was later being weaned once his symptoms were controlled. In view of previous history of GVHD, GI consultation was sought and he underwent UGI endoscopy and biopsy which was

non-significant. On Day 7 of his admission he developed a pustular skin lesion on thigh and scrapping from that showed VZV and his blood PCR was also positive, he was started on intravenous acyclovir. His lesions improved and crusted and his abdominal pain subsided after 72 h of acyclovir and was discharged on oral acyclovir after 14 days of intravenous therapy. Review of literature illustrated in Table 1. Severe abdominal pain in patients who received an allogeneic stem cell transplant has a broad differential. Here we describe a case of VZV presenting with intractable abdominal pain needing opioids. Because of the poor prognosis and life-threatening nature of disseminated VZV disease, it should be considered and included in the patient's workup.

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References	Sex	Age (yrs.)	Disease	SCT type	Post SCT(mont hs)	VZV diagnosis (lag in days)	outcome
1	F	61	NHL	Autologous	13	10	Died day11
2	M	38	CML	Allogeneic unrelated donor	12	10	Recovered
2	F	32	ALL	Allogeneic sibling donor	14	7	Recovered
3	M	57	CML	Allogeneic sibling donor	6	9	Recovered
4	M	35	CML	Allogeneic sibling donor	5	10	Died day11
5	N.A	41	AML	Allogenic Sibling	11	No skin lesion, Post mortem	Died
6	M	30	CML	Allogenic sibling	11	5	Died day 4
6	M	32	ALL	Allogenic sibling	12	4	Recovered
Present case	M	13	ALL	Allogeneic unrelated donor	4	7	Recovered

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**Disclosure of conflict of interest:** None.

## P269

### Intravesical cidofovir application in BK-virus cystitis after allogeneic hematopoietic stem cell transplantation (HSCT) is safe and highly effective

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BK virus (BKV) is a double-stranded DNA polyomavirus with ~90% seroprevalence. BKV cystitis is a potentially life-threatening complication early after allogeneic stem cell transplantation (HSCT) leading to dysuria and hemorrhagic cystitis (HC). There is no standardized treatment. Most recommendations suggest intravenous (i.v.) cidofovir therapy, which induces significant toxicities. We performed a retrospective monocentric analysis of BKV cystitis in patients undergoing HSCT from 2012 to 2016 with a special focus on efficacy and safety of 1st line intravesical cidofovir therapy. All HSCT patients between October 2012 and October 2016 were retrospectively screened for BK cystitis. Urine BKV testing was initiated with PCR in case of dysuria or hematuria after HSCT and exclusion of bacterial urinary tract infection. Hematuria was graded according Bedi *et al.* (grade 1°–4°). Intravesical cidofovir (5 mg/kg, diluted in 90 ml sterile water) was once weekly applied until symptom control for 60 min. via a transurethral catheter, i.v. cidofovir was initiated if no symptom control was achieved after 3 local applications. In patients with HC 3 or 4 a lavage catheter was added. BKV cystitis (dysuria ( $n=8$ ) or dysuria combined with hematuria ( $n=10$ )) developed in 18 out of 152 transplants (12%). Median age was 54 years, 89% were female and 50% received a mismatch transplantation after 1 MAC or 17 RIC conditioning regimens. In 67% of BKV cystitis cases also CMV reactivation within the first 180 days could be detected. 67% had acute GvHD II°–IV° at the onset of BKV cystitis and 83% received steroid medication. The median time to symptom occurrence was day +40 after HSCT (IQR 25–75: 31–136). 5 patients (3 with dysuria and one either HC 1° and 2°) didn't require therapy due to self limiting symptoms. 3 (23%) of 13 treated patients showed only dysuria, 3 (23%) HC 1°, 5 (38%) HC 2°, 1 (8%) HC 3° and 1 (8%) HC 4°. The first patient was treated with i.v. cidofovir twice and symptoms relieved. All the following 12 patients were exposed to intravesical cidofovir as 1st line therapy. 8 patients (67%) achieved a complete remission with a median of 2 intravesical procedures (range: 1–3). 1 patient showed symptom improvement and all 9 patients didn't require further therapy. 3 patients had to be switched to i.v. application due to bladder spasms during intravesical application ( $n=1$ ) or to insufficient symptom control ( $n=2$ ). 2 out of these 3 responded to i.v. treatment, whereas 1 patient receiving 2nd transplant didn't respond at all. In patients with spontaneous symptom relieve the median BKV concentration at the time of symptom onset was 2 log lower compared to those requiring antiviral therapy. Local therapy reduced BKV

viruria by 2 log. Pain during cidofovir instillation in 50% of patients was the only significant side effect of local therapy compared to creatinine increases by >50% in 66.6% of i.v. treated patients. Intravesical treatment of symptomatic BKV cystitis with cidofovir (5 mg/kg) is safe and effective with an 75% symptom improvement rate and no systemic side effects. In patients without sufficient symptom or bleeding control i.v. cidofovir is still an option, which however induces significant renal toxicity. We therefore recommend intravesical cidofovir as 1st line therapy in case of dysuria or hematuria induced by BKV after HSCT.

**Disclosure of conflict of interest:** None.

## P270

### Intravesical cidofovir is an effective treatment of BK-virus positive haemorrhagic cystitis in post stem cell transplant patients: experience in Leeds Children's Hospital, UK

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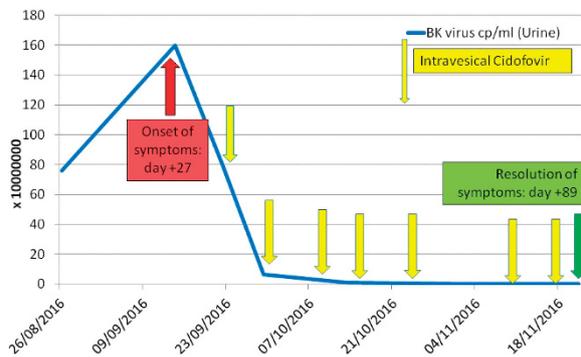
Haemorrhagic cystitis is a recognised complication of stem cell transplant (SCT), with a reported incidence of 5–25% of cases (1). The majority of cases are associated with BK polyomavirus (BKV), and less often adenovirus and cytomegalovirus. There are a lack of high quality studies on the optimal prevention and management of haemorrhagic cystitis. Treatment options are restricted by conditioning toxicity, immunosuppression and other co-morbidities such as renal impairment. Cidofovir has an inhibitory effect on BKV replication and has been used extensively in the treatment of haemorrhagic cystitis. However, severe nephrotoxicity limits routine intravenous use in SCT patients. Alternative options include using low dose intravenous cidofovir or intravesical administration. We conducted a retrospective case review of post SCT patients presenting with BK virus associated haemorrhagic cystitis in our institution between January 2010 and November 2016. We identified 5 patients in total (4 male, 1 female). The indications for stem cell transplant were as follows: severe aplastic anaemia 1 high risk AML 1 relapsed AML 1 relapsed ALL 2 onset of symptoms (haematuria and painful micturition) ranged from day -4 to day +27, and the time to resolution of symptoms varied from 16 days to 68 days. Four of the patients were treated with intravesical cidofovir only, with the number of doses required varying from 3 to 7. One patient received combination treatment with both intravenous (7 doses), and intravesical cidofovir (5 doses). All patients had a good clinical response with complete resolution of symptoms and no major complications. However, the level of BK virus in the urine did not always correlate with clinical response. Some of the patients did not tolerate urethral catheterisation and required a general anaesthetic for the placement of the urethral catheter; 1 patient required a supra-pubic catheter. Currently 3 out of 5 patients are alive and well; 2 patients died from causes not related to BK virus associated haemorrhagic cystitis. Our experience shows that intravesical administration of cidofovir is a safe and effective option for the treatment of BK virus associated haemorrhagic cystitis.

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[P270]

Patient 5 (SC): Matched sibling SCT for high risk AML



Disclosure of conflict of interest: None.

P271

Previously published

P272

**Multiple peptide vaccination against cytomegalovirus (CMV) elicits immunological and clinical responses after allogeneic stem cell transplantation even from a CMV seronegative donor**

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An allogeneic stem cell graft from a cytomegalovirus (CMV) seronegative donor puts recipients at high risk of CMV reactivation which can lead to CMV disease and mortality. Based on the immunogenicity of CMV phosphoprotein 65 (CMVpp65) we initiated a clinical phase I trial with a novel vaccine designed by our group: a CMVpp65-derived peptide in water-in-oil emulsion (Montanide) plus administration of granulocyte-macrophage colony stimulating factor. Ten patients received four vaccines s.c. at a biweekly interval after allogeneic stem cell transplantation. We monitored the patients for their clinical outcome and CMVpp65 antigenemia. Multi-color flow cytometry test were performed to assess CMV-specific CD8+ and gamma-delta T cells. Novel neutralizing anti-CMV antibody assays were established and correlated to clinical parameters. Findings: In general, patients tolerated the peptide vaccination well, no drug-related adverse events others than rash or induration at the site of injection were detected. Seven of nine patients with CMVpp65 antigenemia cleared the CMV after four vaccinations and were hitherto free from antigenemia. Two patients with CMV reactivation showed persisting CMV antigenemia. One of these two refractory patients received additional four injections and

remained hitherto free from CMV antigen. Another patient obtained a prophylactic vaccination and did not develop antigenemia. An up to six-fold increase in frequency of both CMV-specific CD8+ T cells or Vdelta2-gamma-delta T cells was detected in five patients. Moreover, titers of neutralizing antibodies increased in four patients up to 10-fold over the time of vaccination. Humoral and cellular immune responses correlated with clearance of the CMV load. CMVpp65 peptide vaccination was safe and well tolerated in patients after allogeneic stem cell transplantation at high risk for CMV reactivation. The vaccine showed encouraging immunological and clinical results. A prophylaxis study using the vaccine in solid-organ transplant patients is ongoing.

Disclosure of conflict of interest: None.

P273

**Necrotic lymph-node infection with sporopachydermia cereana in a patient with acute myeloid leukemia: a case report**

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*Sporopachydermia cereana* is a rare yeast found in necrotic cactus tissue, predominantly in the Americas. Infection in humans has only been reported in 4 neutropenic patients with fatal course, either directly from the pathogen or other complications of immunosuppression. Treatment is complicated by difficulties in pathogen-identification with conventional diagnostic techniques and by resistance to echinocandins. Here we present a patient with acute myeloid leukemia (AML) and *S. cereana* infection. This is the first patient who was successfully treated with antifungal therapy and who survived *S. cereana* infection. Case presentation We present the case of a 50-year-old female patient who was diagnosed with normal karyotype AML with DNMT3A and IDH2 mutations in December 2015. She achieved complete remission after two cycles induction chemotherapy. During the 2<sup>nd</sup> induction cycle the patient developed persistent fever in neutropenia despite broad-spectrum antibiotics and the replacement of prophylactic fluconazole to caspofungin. Blood cultures showed growth of *S. cereana*, shown to be sensitive to azoles (MIC fluconazole < 1 mg/L, MIC voriconazole < 0.12 mg/L) as well as amphotericin B (MIC < 0.25 mg/L), but resistant to caspofungin (MIC > 4 mg/L). Following the susceptibility profile the treatment was changed first to liposomal amphotericin B, and with the availability of MIC results to voriconazole. Metastatic fungal infection (that is, endocarditis, endophthalmitis, hepatosplenic candidiasis) was excluded. After regeneration of peripheral blood values the treatment was switched to oral voriconazole. A CT scan of the chest and abdomen prior to allo-HSCT after 6 weeks of treatment with voriconazole revealed new multiple necrotic mesenteric lymph nodes. An ultrasound-guided biopsy of a node revealed no growth on fungal cultures, a Grocott stain revealed no hyphae or spores. A panfungal PCR of an ITS (Internal transcribed spacer) fragment revealed fungal DNA, which could be confirmed as *S. cereana*. At this time the level of voriconazole in serum was found to be sub-therapeutic (0.4 mg/L), and the dosage was increased accordingly. Subsequent CT scans 4 and 6 weeks later revealed a regression of the affected abdominal lymph nodes. In the further course non-myeloablative conditioning with fludarabine and busulfan prior to allo-HSCT using PBSC

from her HLA-matched brother was performed. Under prophylaxis with cyclosporine, methotrexate and anti-thymocyte globulins (ATG) graft-versus-host disease (GvHD) remained absent. The allo-HSCT was performed under voriconazole treatment with no further complications and the patient engrafted at day 20. The treatment was changed to fluconazole 400 mg daily before discharge. Due to the complete radiological regression of the infection in follow-up scans and excellent general condition of the patient 5 months after HSCT, fluconazole was discontinued. The patient remains in morphological complete remission 6 months after HSCT and has a 100% donor chimerism. The first published case of survival of infection with *S. cereana* exemplifies the continual progress made in treating infections in the severely immunocompromised patient. Diagnosis via ITS sequence-analysis seems reliable but a high index of suspicion is required for neutropenic patients who do not respond well to standard antimycotic therapy. The increased availability of the technology may lead to more frequent diagnoses in the future.

**Disclosure of conflict of interest:** None.

**P274  
Neutropenic enterocolitis incidence after autologous stem cell transplantation**

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Neutropenic enterocolitis (NE) is a clinical syndrome characterized by fever and abdominal pain in patients who received chemotherapy for hematological malignancies and who treated with stem cell transplantation (SCT). The aim of this study was to determine the incidence, risk factors and outcome of NE after

autologous SCT (auto-SCT). We retrospectively evaluated 226 patients with Non-Hodgkin Lymphoma (NHL), Hodgkin lymphoma (HL) and multiple myeloma (MM) who underwent auto-SCT between January 2013 and December 2016 in our center. Patients with lymphoma were conditioned with carmustine, etoposide, cytarabine, melphalan (BEAM) or thiotepa, etoposide, cytarabine, cyclophosphamide, melphalan (TECAM). Patients with multiple myeloma were treated with melphalan as conditioning. Diagnosis of NE was established in case of neutropenic fever, abdominal pain or diarrhea, and bowel wall thickening >4 mm on abdominal ultrasonography. Febrile neutropenia was seen in 199 (88%) patients of all. The median time from transplantation to neutropenia was 4.5 days (range: 0–9 days). NE occurred in 22 (9.7%) in all neutropenic patients. The median time to NE after auto-SCT was 7 days (range: 2–9 days). The median neutrophil engraftment time was 12.5 days (range: 9–18 days). Abdominal pain was seen in all patients with NE. Twenty one patients (95%) had diarrhea. Ileus was seen in 1 (4.5%) patient and septic shock was developed in 3 (13.6%) patients. Five (22.7%) of 22 patients had bloodstream infection. *Klebsiella pneumoniae* in 1, *Pseudomonas aeruginosa* in 1, *Escherichia coli* in 1, *Staphylococcus aureus* in 1 and coagulase-negative staphylococcus in 1 patient were documented in patient's blood stream. Early diagnosis was made by abdominal ultrasonography in all patients at a day of median 7 days (range: 2–9). Twenty (91%) patients were resolved completely with good supportive care and proper antibiotherapy. Two (9%) patients died of septic shock and ileus.

NE is a rare but serious complication in patients underwent high dose chemotherapy followed by auto-SCT. Gram-negative bacteria are the main causative pathogens. Abdominal ultrasonography is the simple, cheap, fast diagnostic and noninvasive procedure that allows the early diagnosis and effective treatment.

**Disclosure of conflict of interest:** None.

[P274]

**Table 1: Patients' characteristics**

Characteristics	N=226	Patients with NE (N=22)	Patients without NE (N=204)	Statistics
Sex (male/female)	127/ 99	15/7	112/ 92	NS
Median age at transplantation, years	56 (20- 77)	57.5 (22- 65)	56 (20- 77)	NS
MM (%)	129 (57.1)	5 (22.7)	124 (60.8)	p=0.001
HL / NHL (%)	97 (42.9)	17 (77.1)	80 (29.2)	p=0.001
Conditioning regimen (BEAM/ TECAM/ Melphalan) (%)	64 (28.3)/ 33 (14.6)/ 129 (57.1)	13 (60)/ 4(18.2)/ 5 (22.8)	51(25)/ 29 (14.2)/ 124 (60.8)	p=0.001
Febrile neutropenia (yes/no) (%)	199 (88)/ 27 (12)	22 (100)	177 (86.8)/ 27 (13.2)	NS
Neutropenic enterocolitis (no/ yes) (%)	204/ 22 (9.7)	22	0	

**P275****Neutrophil transfusions in the treatment of neutropenic patients submitted to allogeneic HSCT: possible role on graft failure**

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Granulocyte transfusions (GTx) from G-CSF-stimulated donors have been shown to increase the absolute neutrophil count (ANC) before expected haematopoietic recovery in neutropenic patients after chemotherapy or haemopoietic SCT. Thus GT offers a therapeutic option along with antimicrobial agents and growth factors to improve clinical outcome of neutropenic patients with severe infections. The primary limitations of GT include low component cell dose and leukocyte incompatibility. The transfusion of G-CSF-mobilized, HLA-matched granulocyte components resulted in sustained ANC increments, but the efficacy of this procedure has not been established by convincing randomized control trials. AIM: We focused our attention on GT in the setting of allogeneic HSCT, in particular on the feasibility and safety of this procedure on the rate of engraftment. Between 2006 and 2016 our centre performed 211 allogeneic HSCT. We analyze data from 59 transplanted patients receiving GT at some point during their disease. Indication for GT was severe sepsis mainly due to MDR GRAM-bacteria. Patients received a median of 4 GT (1-34), in different phase: 24 patients during induction therapy, 18 during HSCT, 9 at diagnosis and during HSCT and 8 after HSCT. Patients' characteristics are summarized in Table 1. Median

[P275]

<b>Patients</b>	59
<b>M/F</b>	35/24
<b>Median age (range)</b>	42 (14-65)
<b>Disease</b>	AML 41 ALL 9 MDS 1 Lymphoproliferative Disease 9
<b>Disease status at tx</b>	CR 27 Refractory/relapse 25 PR 4 Frontline 3
<b>Conditioning regimen</b>	MA 34/RIC 25
<b>GvHD prophylaxis</b>	CSA+MTX 39 CSA+MMF 15 Other 4
<b>Median CD34+ dose (range)</b>	6.4x10 <sup>6</sup> /kg (1.2-24)
<b>Donor type</b>	Sibling 23 MUD 30 Cord blood 4 Haploidentical 3
<b>Median Neutrophil engraftment (range)</b>	16 d (7-36)
<b>Median Platelets engraftment (range)</b>	14 d (2-54)
<b>Sepsis/FUO</b>	36/14
<b>Relapse</b>	21/59
<b>OS</b>	22/59

CD34+ cells dose was 6.4x10<sup>6</sup>/kg (range: 1.2-24). Donor source was in 49 patients G-CSF mobilized peripheral blood, 6 bone marrow and 4 cord blood. Median neutrophil recovery (>500/mm<sup>3</sup>) was 16 days and platelet recovery (>20 000/mm<sup>3</sup>) was 14 days. Sepsis were documented in 36 pts and 45 pts developed FUO. Relapse was documented in 21 pts (35%). Twenty-two pts are still alive and in complete remission (37%), Death occurred in 37 pts: 19 due to TRM and the remaining 18 for disease relapse. Graft failure occurred in 16 of the 211 pts submitted to HSCT. Among the 11 patients (18%) who experienced graft failure, six (54%) received GT before HSCT, because of sepsis during the induction therapy, and the remaining 5 after HSCT, during aplasia period. In the remaining group (152 pts) not receiving GT, only 5 (3%) graft failure were observed. Thus a statistically difference ( $P=0.0005$  Fisher's exact test) increase in the rate of graft failure was detected in patients receiving GT. The role of GT in the treatment of infections in neutropenic patients remain still unclear for several reasons including the lack of clinical trials convincingly and consistently demonstrating efficacy, by availability of GT donors and by center's experience. GT has been successfully used in our center in patients with severe sepsis from MDR GRAM-bacteria during severe neutropenia but an increase number of graft failure has been registered in patients subsequently receiving HSCT. Alloimmunization to HLA antigens in patients receiving GT might lead to an excess of graft failure requiring HLA antibodies detection and attempt to reduce titer prior to HSCT and maximizing stem cell dose.

**Disclosure of conflict of interest:** None.

**P276****Oral health status and risk of bacteremia in patients undergoing myeloablative allogeneic hematopoietic cell transplantation**

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Viridans streptococci are microorganisms frequently isolated from blood cultures of patients undergoing myeloablative allogeneic hematopoietic cell transplantation (alloHCT). Poor dentition status has been associated with an increased risk of streptococcal bacteremia in the immediate post-alloHCT neutropenic period. The objective of this study was to evaluate the impact of oral health status on bacteremia risk in a cohort of patients undergoing therapy for acute myeloid leukemia (AML). A retrospective study was conducted in patients with AML treated at Dana-Farber/Brigham and Women's Cancer Center (DF/BWCC) from 2007 to 2011. There was no formal dental assessment prior to AML induction therapy. All patients underwent protocol directed pre-alloHCT dental evaluation that included a standardized examination, comprehensive dental radiographs, and detailed treatment planning guidelines. Poor oral health status was defined as presence of acute or chronic odontogenic infection, and it was assumed that oral health status at the time of induction therapy was the same as the pre-alloHCT evaluation findings. Oral health status at the time of alloHCT was determined by the completion of required dental treatment. Positive blood cultures were recorded from AML induction to day +60 post alloHCT. Organisms that caused bacteremia were classified as 'of possible oral source' by a blinded microbiologist. Two-sided Fisher's exact test was used to compare the oral health status of the entire cohort to patients with blood cultures of potential oral source. From January 2007 to January 2011, 181 patients with AML underwent myeloablative alloHCT at DF/BWCC and were

followed through today +60, and of these, 92 patients met the inclusion criteria and were included in the cohort. The median age was 48 years (range: 24–66) and there was similar distribution of genders. The most common AML induction regimen was daunorubicin and cytarabine (63/92; 68%) and of those that received consolidation therapy (49/92; 53%), almost all patients were treated with cytarabine. Nearly all patients (90/92; 98%) received cyclophosphamide and total body irradiation for alloHCT conditioning and the majority of patients (83/92, 90%) received tacrolimus/methotrexate ( $n=51$ ) or tacrolimus/sirolimus ( $n=32$ ) for GVHD prophylaxis. Over half of patients (51/92, 54%) experienced mucositis during their course of therapy for AML. Pre-alloHCT dental evaluations were completed in 91/92 (99%) of patients. Of the 13/91 (14%) patients identified as having poor oral health status, 13/13 (100%) completed all required dental treatment prior to alloHCT. Bacteremia occurred in 63/92 (68%) patients, and 12/63 (19%) had positive blood cultures of potential oral source. Of the 12 patients with positive blood cultures of potential oral source, 1/12 (8%) patient developed bacteremia during induction and 11/12 (92%) patients developed bacteremia during alloHCT. Of the 13/91 (14%) patients identified as having poor oral health status, one patient (1/13; 8%) had a positive blood culture with a bacteria of potential oral source during induction/consolidation ( $P=0.68$ ). Oral health status was not associated with risk of bacteremia of potential oral source at either AML induction/consolidation or alloHCT. Risk of such bacteremia in the setting of myeloablative alloHCT may be related more to overall gastrointestinal translocation.

**Disclosure of conflict of interest:** None.

**P277**

**Poor absorption of Atovaquone in stem cell transplant patients: a cause of failure of pneumocystis prophylaxis?**

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In immunocompromised patients, including in hematopoietic stem cell transplant (HSCT) recipients, Atovaquone (ATO)

is one of the main alternatives to trimethoprim-sulfamethoxazole (TMP-SMX) for prophylaxis of pneumocystis pneumonia (PCP)(Maertens *et al.* JAC 2016). ATO is less effective than TMP-SMX to prevent PCP1 but the reasons of this lower efficacy are not well understood. ATO acts on Pneumocystis, Plasmodia and Toxoplasma species by inhibiting mitochondrial pyrimidine biosynthesis. ATO is highly lipophilic and its absorption in volunteers is improved by a fatty meal. There is a wide inter-individual variability in bioavailability and many drug interferences. The aim of this study was to assess the plasma concentrations of ATO in patients under PCP prophylaxis with ATO oral suspension and explore the factors which might impact its bioavailability. All adult patients receiving ATO for PCP prophylaxis in the hematology and clinical immunology wards between May and September 2016 were included in the study. The prescribed dose was 750 mg of oral suspension twice a day. Blood samples were collected around 12 h after the evening dose (Cmin) and 1–5 h after the morning dose (Cmax). Plasma was immediately separated after each sample and frozen at -20 °C until proceeding to the assay. ATO plasma levels were measured by UV-high-performance liquid chromatography. Clinical and biological data, exact timing and modalities of intake (during a meal or not), and concomitant medications were collected. Cmin and Cmax results are presented as median (IQR 25–75%) and compared by Mann–Whitney *U*-test or signed rank test when appropriate. Patients: A total of 85 measurements were performed in 33 patients (allogeneic HSCT patients: 19; hematology non-transplanted patients: 6; HIV-infected patients: 7). The mean age (range) was 53 years (33–75), the M/F ratio was 21/12. Only two patients were neutropenic. The median Cmin was 11.3 µg/mL (6.2–27.8) and the median Cmax was 13.4 µg/mL (6.0–28.3). Thirteen of the 33 (39%) patients had a Cmin.

**Disclosure of conflict of interest:** None.

**P278**

**Presepsin as a marker of infectious complications during high-dose chemotherapy following autologous hematopoietic stem cell transplantation in lymphoma patients**

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Lymphoma patients, who undergo high-dose chemotherapy following autologous hematopoietic stem cell transplantation (autoHSCT), are at high risk of developing infectious complica-

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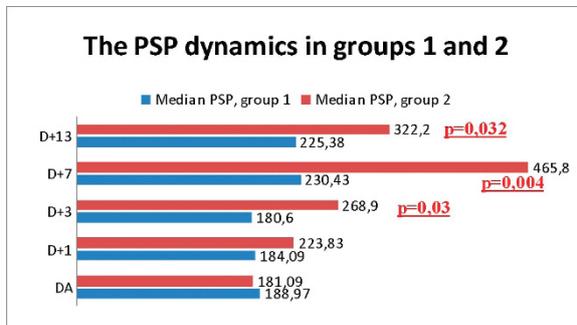
**Table 1. The dynamics of presepsin on the days of assessment between groups 1 and 2**

Days of assessment	Median PSP, group 1	Median PSP, group 2	t-value	p	N	N
DA	188.97	181.09	0.341	0.73	31	67
D+1	184.09	223.83	-1.3	0.194	31	69
D+3	180.6	268.9	-2.19	<b>0.03</b>	27	64
D+7	230.43	465.8	-2.95	<b>0.004</b>	31	69
D+13	225.38	322.2	-2.17	0.032	31	69

Unlike PSP, in the analysis of PCT significant differences between groups were determined on the DA and on D+1. On D+3, D+7 and on the DD statistically there were no significant differences between the analyzed groups. In the analysis of C-RP there were significant differences on D+3 and D+7. Differences on D+3 can point to the prognostic value of C-RP. Analysis of dynamics of biochemical markers in lymphoma patients undergoing autoHSCT has shown that PSP has greater value in diagnosis and prognosis of infectious complications.

tions (IC). Mortality from IC during the transplantation, according to various data ranges from 12 to 42%. Thus the development of models of early prognosis of IC during autoHSCT has become more urgent. It's reasonable to include the dynamics of biochemical markers of inflammation in these models. Presepsin (PSP), procalcitonin (PCT) and C-reactive protein (C-RP) were assessed on the day of admission to the hospital (DA), on D+1, D+3, D+7 and on the day of discharge (DD). If patients developed neutropenic fever (NF), the markers were assessed at the beginning of the fever, 6 h after, then on the second, third, fourth days after. There were 100 patients included in the study: 41 patients with Hodgkin lymphoma, 27 – with non-Hodgkin's lymphoma, 32 – with multiple myeloma, out of 100 patients there were 51 women and 49. The median age was 41 years (18–66). The conditioning regimens were CBV, BeEAC or HD melphalan. Depending on the presence of IC, the patients were divided into 2 groups: group 1 – patients without infectious complications ( $n=31$ ), group 2 – patients with the development of infectious complications ( $n=69$ ). The median of the NF development was 5.5 days. 53 patients from group 2 had no microorganism growth in blood stream, either in repeated studies. Gram+ flora was detected in 12 patients, 1 patient had Gram-, 2 patients had mixed flora and 1 patient had *Pneumocystis jirovecii* infection with respiratory insufficiency grade 3. Significant differences in PSP level between groups 1 and 2 were determined on D+3, on D+7 and the DD after autoHSCT. Considering the median day of the NF appearance (5.5 days), it's supposed both the prognostic value (differences on D+3, that is, 2 days before the clinical manifestation of infection) and the diagnostic value of PSP (differences on D+7 and on the DD) (Table 1, Graph 1).

[P278]



**Disclosure of conflict of interest:** None.

**P279**

**Prospective analysis of BK viremia: increases hemorrhagic cystitis (HC) incidence in allogeneic hematopoietic stem cell transplantation (Allo-HSCT)**

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HC is often a serious complication and occurs in 70% of allo-HSCT recipients. Early bleeding is usually the result of chemotherapy toxicity however late occurring HC is multifactorial. BK virus infection has been shown to be related with HC. Most studies demonstrate BK virus at the time of bleeding therefore not allowing the risk imposed by asymptomatic infection to be estimated. In this study, our aim is to show the effect of risk factors as well as pre-transplant BK viral load in asymptomatic recipients on development of HC in allo-HSCT.

Between 2014 and 2016, we prospectively evaluated 59 allo-HSCT. In order to detect the BK viral load, we performed quantitative BK virus PCR (Altona Diagnostics, Germany) from blood samples at days 0, 30, 60 and 90 after allo-HSCT. Informed consents were obtained from all participants. BK virus PCR was considered positive if any number of copies were detected above the analytical sensitivity of the tests. The patients were monitored for signs and symptoms of HS. The risk factors for the development of HS were evaluated by univariate and multivariate analysis.  $P < 0.05$  was considered statistically significant. The median age of the group was 41 (range: 22–71), 18 of the patients (31%) were aged  $> 50$ . Male to Female ratio was 1.36 (34/25). Fifty two patients (88%) had diagnosis of malign hematological disease. Stem cell source was peripheral blood in 51 (86%), bone marrow in 8 (14%) allo-HSCT. Patients received stem cells from 26 related donors (44%) vs 33 (56%) unrelated or haplo donors. Myeloablative conditioning was administered in 47 patients (80%). Forty-four of the conditioning regimens (75%) included cyclophosphamide. HC was diagnosed in 22 patients (37%) at a mean of 100 days (range: 0–367), early HC was detected in 4 of 22 patients (18%). The frequency of BK viremia and number of viral copies are given in detail in Table. The frequency of BK viremia increases during transplantation in relation to clinical HC (66%, 66%, 87%, 100%;  $P=0.007$ ). Acute graft vs host disease (aGVHD) was diagnosed in 37 patients (63%) at a median time of posttransplant day 67: Grade I–II Gastrointestinal/Skin/Liver in 31 (84%), Grade III–IV Gastrointestinal/Skin/Liver in 6 patients (16%). The most common GVHD prophylaxis preferred was cyclosporine and methotrexate in 50 patients (85%). In univariate and multivariate analysis (age  $> 50$ , sex, diagnosis, stem cell source, donor type, conditioning regimen, aGVHD, Cy administration, BK virus PCR at days 0, 30, 60) BK virus titer positivity at day 0, 30, 60 ( $P=0.008$ ,  $P < 0.001$ ,  $P < 0.001$ ), myeloablative conditioning ( $P=0.018$ ), the presence of aGVHD after day 30 ( $P=0.018$ ) and conditioning regimen that includes cyclophosphamide ( $P=0.024$ ) are found to be related with increased risk of HS. Patients with HC and clots were treated with continuous bladder irrigation as well as 8 of patients with BK viremia received cidofovir and six of them responded to treatment (75%). Our study showed that, BK titer positivity, myeloablative conditioning, presence of aGVHD, cyclophosphamide containing conditioning are associated with HC. Detection of BK viremia in later transplant period is more sensitive for clinically proven HC. Prophylactic treatment might be considered in patients with asymptomatic BK viremia in pretransplant period.

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Table. BK viremia and number of viral copies, \* $P=0.007$

<b>DAY 0</b>	
BK virus + patients (n,%)	15 (25%)
Mean number of BK viral copies/ml (range)	10,949 (23-614,608)
*HC in transplant period (n,%)	10 (66%)
<b>Day 30</b>	
BK virus + patients (n,%)	24 (41%)
Mean number of BK viral copies/ml (range)	2,604,813 (10-37,869,641)
*HC in transplant period (n,%)	16 (66%)
<b>Day 60</b>	
BK virus + patients (n,%)	16 (27%)
Mean number of BK viral copies/ml (range)	7,785 (16-267,145)
*HC in transplant period (n,%)	14 (87%)
<b>Day 90</b>	
BK virus + patients (n,%)	7 (12%)
Mean number of BK viral copies/ml (range)	302,353 (5-5,371,515)
*HC in transplant period (n,%)	7 (100%)

**Disclosure of conflict of interest:** None. This project has been granted by Ankara University Scientific Research Committee numbered as 15B0230007.

**P280****Prospective study comparing neutropenic enterocolitis in 147 lymphoma patients transplanted with beam vs feam: the role of ultrasound on incidence, diagnosis, treatment and outcome in this life threatening complication**

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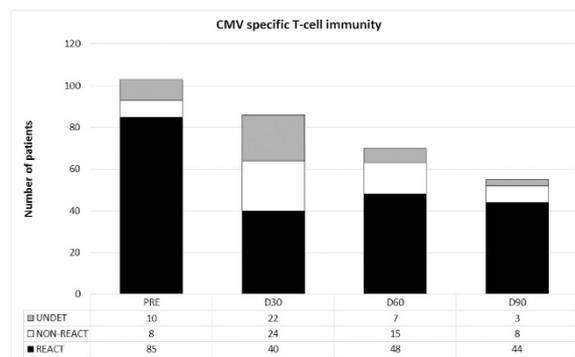
High-dose chemotherapy with peripheral blood progenitor cell (PBPC) collection followed by a myeloablative conditioning and autologous stem cell transplantation (ASCT) is considered the standard of care of relapsed/refractory non Hodgkin/Hodgkin lymphoma (NHL/HL). A widely adopted conditioning regimen is the combination of carmustine etoposide cytarabine and melphalan (BEAM), whose feasibility and efficacy has been largely demonstrated. High dose fotemustine plus etoposide, cytarabine and melphalan (FEAM) has in some cases replaced BEAM conditioning. Neutropenic enterocolitis (NEC) is a life threatening complication of patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50%. It's a clinical syndrome in neutropenic patients (pts) characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and >4 mm is considered diagnostic of NEC. Early diagnosis is crucial to start conservative medical management (CMM), which appears the optimal strategy for most cases. **Objective:** 1. to evaluate if NEC incidence and outcome differs in BEAM vs FEAM and 2. To evaluate prospectively if Bed-side-US (BUS) can detect early signs of NEC and guide a prompt treatment (CMM or surgical) in order to reduce mortality. In the last 5 years all pts with NHL/HL admitted in our BMT Unit wards at University of Pisa (Italy), undergoing ASCT were prospectively enrolled. Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12 h from onset: F and/or D and/or AP in CHT-related neutropenic pts. 95 pts were conditioned with BEAM and 52 pts with FEAM. NEC was diagnosed in N=19/52 FEAM and in N=25/95 BEAM patients. Incidence was 36% and 25% respectively, without a statistically significant difference (P=0.234). Two pts died/19 in FEAM arm (10.5%) and 2pts/24 in BEAM arm (8.3%), without a statistically significant difference (P=0.778). At time of diagnosis (Dx) symptoms were: F+AP+D 45%, F+D 4%, F+AP 3%, AP+D 35%, D 3%, AP 10%. F alone was never present at diagnosis of NEC. At Dx, F was absent in 18/44 NEC episodes (40%). All pts were treated promptly as BUS allowed diagnosis with CMM except one 1 pts who underwent surgery, guided by US features, during neutropenia. The likelihood of NEC Dx in a discriminant St model (Bayes theorem) for pts with BWT and AP=98.8%, AP+D=99.9%, AP+D+F=100%, AP+F=99.9%, D+F=5%. BUS allowed to detect early signs of NEC and to start prompt treatment in this life threatening complication, of NHL/HL pts undergoing ASCT. This is a prospective study thus the true incidence of NEC in NHL/HL undergoing ASCT should not be underestimated. There is not a statistically significant difference in incidence and outcome of NEC in pts conditioned with BEAM in respect to FEAM. With BUS pts do not live the isolation room. Fever is not a condition sine qua non for NEC diagnosis. Early diagnosis allows most of pts to be treated with CMM. Images of BUS and CT were superimposable with lower costs, and less radiation exposure. A low mortality rate in pts with a 25–36% chance of developing this life threatening complication suggests that a prompt BUS in neutropenic patients as just one symptom presents allows to make early diagnosis of this life threatening complication and guide prompt treatment (conservative or surgical), reducing mortality. **Disclosure of conflict of interest:** None.

**P281****Quantiferon-CMV in the evaluation of CMV-specific immunity after autologous and allogeneic HSCT**

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Cytomegalovirus (CMV) is a major cause of morbidity and mortality after allogeneic HSCT. The same is not observed in autologous HSCT recipients who do not need to receive immunosuppression after transplantation. In the present study, we compared the reconstitution of CMV-specific immunity in autologous and allogeneic HSCT recipients. Patients were invited to participate in the study and signed the informed consent. CMV surveillance with the antigenemia (AG) test (CMV brite, Biotest, Germany) was done weekly in the first 3 months of transplant in allogeneic HSCT recipients. Pre-emptive ganciclovir therapy was initiated whenever a positive antigenemia was detected. The presence or absence of CMV-immunity was determined by a commercial interferon (INF) gamma release assay (Quantiferon-CMV, Qiagen) before HSCT and monthly thereafter up to d+90. From January to October 2016, 106 HSCT recipients (29 auto and 77 allo) were included in the study. AG was positive in 54 (70%) of the alloHSCT recipients at a median of 39 (range: 14–146) days. AG recurrences occurred at a median of 81.5 (38–249) days, in 15 of the 54 pts (27.8%) who had at least one episode of positive AG. 103 HSCT recipients were included in the analysis of QTF-CMV. In the pre-HSCT sample, QTF-CMV was reactive in 60 of the 85 alloHSCT (70.6%) and in 25 of the 28 autoHSCT (89.3%). Significantly less allo HSCT recipients recovered CMV-immunity at day +30 (31.8%) and day+60 (59.3%) in comparison with autoHSCT (95% and 100%, respectively, P<0.01). Up to day +90, all autoHSCT have recovered CMV-immunity, in comparison to 68% of the alloHSCT recipients (P=0.096, figure 1). The QTF-CMV test performed at d+30, d +60 and d+90 did not predict the risk of CMV reactivation in the following month. Similarly, the test did not anticipate the risk of AG recurrences: 80% of the HSCT recipients with undetermined or non-reactive QTF-CMV test at d+60 had AG recurrence after this period, in comparison with 70% of the patients with a reactive result (P=0.56). In the present study, the QTF-CMV test alone could not predict the risk of CMV reactivation or recurrences.

[P281]



**Disclosure of conflict of interest:** Qiagen.

**P282****Recovery of Vδ2+ γδ T cells is critical to Epstein-Barr virus reactivation after haploidentical hematopoietic stem cell transplantation**

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Epstein-Barr virus (EBV) reactivation and its related disease are life-threatening complications in patients undergone haplo-identical hematopoietic stem cell transplantation (haploHSCT). Our previous studies found that impaired CD4-CD8- T-cell recovery correlated to the increased occurrence of EBV infection after haploHSCT.  $\gamma\delta$  T cells make up 50-90% of CD4-CD8- T cells in the peripheral blood of healthy donors. Expansion of V $\delta$ 1+  $\gamma\delta$  T cells after HSCT has been reported and this subset could respond against autologous EBV-LCL *in vitro*. Selective activation and expansion of V $\gamma$ 9V $\delta$ 2-T cell could inhibit EBV-LPD development in humanized mice. However, the association of  $\gamma\delta$  T-cell recovery with EBV reactivation after alloHSCT remains unknown. This is a prospective cohort study including 110 consecutive patients who were diagnosed as hematological malignancy and underwent haploHSCT. Recovery of T lymphocyte and a panel of subsets, including CD3+, CD4+, CD8+, CD4-CD8-, TCR $\alpha\beta$ +, TCR $\gamma\delta$ +, V $\delta$ 1+, and V $\delta$ 2+ T cells, were determined by flowcytometry at 30, 60, 90, 180 days after haploHSCT. All recipients and donors were tested negative for EBV DNA in the peripheral blood before transplantation. Recipients were monitored weekly for EBV DNA load until day 100 after transplantation. Recipients with peripheral blood plasma EBV DNA load >1000 copies/mL at least on two consecutive occasions were diagnosed as EBV reactivation (EBV+). EBV- cohort generally represents patients whose EBV DNA load < 1000 copies/mL in peripheral blood. Within 100 days after haploHSCT, 17 of 110 (15.5%) recipients were diagnosed as EBV reactivation. Compared to recipients with negative EBV DNA load, the counts of CD3+, CD8+, and TCR $\alpha\beta$ + T cells were not statistically different in the EBV+ cohort from 30 to 180 days after haploHSCT. In contrast, recoveries of CD4+ and CD4-CD8- T cells in EBV+ patients were significantly hampered at 30 days after transplantation ( $P=0.021$  and  $P=0.046$ , respectively). Although the TCR $\gamma\delta$ + T-cell counts were also decreased at 30 and 60 days in the EBV+ cohort, the comparisons did not reach the statistical significance ( $P=0.072$  and  $P=0.082$ , respectively). Notably, recoveries of V $\delta$ 2+  $\gamma\delta$  T cells at 30, 60 and 90 days were continuously delayed in recipients with EBV reactivation ( $P=0.029$ ,  $P=0.001$  and  $P=0.046$ , respectively). Whereas the counts of V $\delta$ 1+  $\gamma\delta$  T cells were similar between the two groups from 30 to 180 days in this context. In this prospective and large cohort study, we showed that the occurrence of Epstein-Barr virus (EBV) reactivation was associated with the hampered recovery of V $\delta$ 2+ rather than V $\delta$ 1+  $\gamma\delta$  T cells after haploHSCT. Our findings will help explore  $\gamma\delta$  T subset-dependent therapeutic strategies to control the serious complications due to EBV infection post transplantation and improve the overall survival of haploHSCT recipients.

**Disclosure of conflict of interest:** None.

## P283

### Risk factors for pre-engraftment bloodstream infections after hematopoietic stem cell transplantation.

#### Single-centre experience

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Infectious complications are the main problem in patients undergoing Hematopoietic Stem Cell Transplantation (HSCT). In particular, bloodstream infection (BSI) is a frequent complication in the pre-engraftment phase with an impact on the morbidity and mortality of these patients. **Objectives:** To analyze the incidence of BSI in patients undergoing HSCT in our center, and to identify predisposing factors for the development of BSI in pre-engraftment phase patients after HSCT. Fifty-one consecutive patients undergoing HSCT were analyzed retrospectively in our center during the period of 1 July 2015 and 30 June 2016. The characteristics of the sample are shown in Table 1. We have reported all the BSI between day 0 and day 30 after stem cell infusion. 70.5% (36 patients) received antibacterial prophylaxis with ciprofloxacin, five

patients with broad spectrum antibiotics and five did not receive any drug. The average days of fever have been 3.30 days (0-12 days). A total of 184 blood cultures has been collected (3.6 per patient). There have been 15 BSI (21.5% of the patients) with 8 (53.4 %) of cases caused by Gram-negative organism (4 *Escherichia Coli*, *Klebsiella Pneumoniae*, *Acinetobacter baumannii*, *Proteus vulgaris* and *Delftia Acidovorans*) and 7 (46.6%) by Gram-positives (1 *Enterococcus faecium*, 1 *Enterococcus faecalis*, 3 *Staphylococcus Epidermidis*, *Streptococcus mitis* and *Streptococcus viridians* group). One patient presented 3 different episodes of BSI, two patients 2 independent episodes and the rest eight, only one microorganism isolated. We have identified two BSI by Extended-spectrum beta-lactamases (ESBL-producing organism) and one isolation of carbapenem-resistant gram-negative bacteria. The rate of quinolone-resistant is 80% in all the sample. In univariate analysis, several factors like presence of comorbidities, presence of severe mucositis, type of catheter and antibacterial prophylaxis modality don't increased the risk to develop BSI ( $P>0.05$ ). The place where the procedure is performed does not influence the development of BSI. Although the presence of previous infections is not a risk factor, hospitalization for infection in the 90 days before HSCT does influence the development of BSI with statistical significance ( $P<0.05$ ). The crude mortality rate of the sample has been very low (2%), with only one death related to bloodstream infection. BSI are a common relative complication in the patient undergoing HSCT but with an extremely low mortality in our sample. Hospitalization for infection in the 90 days before HSCT does influence the development of BSI. It is important to note that outpatient model and conventional rooms don't increased the incidence of BSI. Although the use of quinolones in prophylaxis does not result in an increase in infections caused by multiresistant micro-organisms (ESBL and carbapenemias) with acceptable resistance rates (80%), it also does not reduce the incidence of BSI in our sample. According to our analysis, his routine employment still throws light and shadows. [P283]

Table 1. Patients Characteristics [

Gender	Male 29 (56.8%)
Age at HSCT (years)	42.3 (17-69)
Diagnostics	
• Acute Leukemia	13 (26%)
• Lymphomas	10 (20%)
• Myelodysplastic Syndromes	2 (4%)
• Multiple Myeloma	18 (36%)
• Chronic Lymphoproliferatives disorders	1 (2%)
• Chronic Myeloproliferatives disorders	1 (2%)
• Aplastic Anemia	4 (8%)
• Lymphohistiocytosis Hemophagocytic	1 (2%)
Comorbidities	
• None	33 (71.7%)
Type of catheter	
• PICC	37 (72.5%)
• Hickman	13 (25.5%)
• Porth-a-Cath	1 (2%)
Type of Room	
• HEPA filter Unit	32 (62.7%)
• Conventional room	10 (19.6%)
• Outpatient	9 (17.7%)
Type of HSCT	
• Autologous	27 (53%)
• Allogeneic	24 (47%)
Conditioning	
• Autologous	27 (53%)
• Myeloablative	12 (23.5%)
• Reduced Intensity Conditioning	10 (19.6%)
• No myeloablative	2 (4%)
N <sup>o</sup> cells CD34+ (10 <sup>6</sup> xuL/Kg)	3.55 (1.95-7.73)

**Disclosure of conflict of interest:** None.

**P284****Septic episodes with multiple bacterial strains during anti-thymocyte globulin (ATG) therapy for conditioning for allogeneic stem cell transplantation under rifaximin gut decontamination**

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Recent evidence demonstrates the importance of the enteric microbiome for the development of gastrointestinal graft-versus-host disease (GvHD) and mortality after allogeneic stem cell transplantation (SCT) (1,2). Accordingly, the usage of the non-absorbed rifamycin derivate rifaximin for gut decontamination has been reported to preserve the intestinal microbiota composition with a positive effect on overall survival in a single centre retrospective analysis (3). We here report severe septicaemia requiring therapy at the intensive care unit (ICU) during ATG application for conditioning in three patients with rifaximin used as single agent for gut decontamination within 6 months. After changing our gut decontamination from a chinolon-metronidazole regimen to rifaximin, three cases of severe septicaemia by gram-negative and gram-positive bacteria during ATG treatment occurred within 6 months. Patient #1 was a 52-year-old woman with tMDS/AML after breast cancer conditioned according to the FLAMSA-Bu protocol. The second (#2) and third (#3) patient were 55- and 37-year-old males with a complex karyotype secondary AML after OMF and relapsed inv(16) AML with meningeosis leucaemica, respectively. Patients #2 and #3 were treated with FLAMSA-Bu and FLAMSA-TBI, respectively. All patients received rabbit ATG (ATG Fresenius/Grafalon) at a dose of 3 × 10 mg/kg body weight and rifaximin (2 × 200 mg) for gut decontamination. Patient #1 developed severe *Escherichia coli* and *Pseudomonas aeruginosa* septicaemia on day -3 of the conditioning regimen and had to be transferred to the ICU with septic cardiomyopathy for therapy with vasopressants and levosimendan. In patient #2 *Escherichia coli*, *Klebsiella oxytoca*, *Staphylococcus hemolyticus* and *Staphylococcus epidermidis* were simultaneously detected in blood cultures at day -2. The patient was transferred to the ICU and treated with vasopressants for septic shock. Patient #3 developed septic shock due to *Klebsiella pneumoniae* and *Enterobacter cloacae* on day -4 under ATG therapy. Mechanical ventilation and vasopressor therapy were required. Fortunately, all three patients survived and completely recovered without any sepsis related disabilities under escalated anti-infective and intensive care therapy. All were discharged from the hospital in the outpatient clinics. Interestingly, all isolated gram-negative pathogens were found to be sensible for a chinolon based gut decontamination. The reasons for these septic complications under ATG therapy are not exactly understood but raise a note of caution on the use of rifaximin as single agent gut decontaminant during ATG application in conditioning for allogeneic SCT.

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**P285****Previously published****P286****Sometimes it is a zebra: rare mycobacterium genavense infection mimicking relapse of T-cell lymphoma after allogeneic hematopoietic stem cell transplantation (HSCT)**

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Infections with mycobacterium genavense were described for the first time in 1990. Since then, several cases have been reported, but almost exclusively in patients with AIDS. Most patients who underwent HSCT have insufficient cellular immunity. Here we report a mycobacterium genavense infection in a patient mimicking a lymphoma-relapse after HSCT. A 58-year-old female patient was diagnosed in July 2013 with Stage IVB ALK-negative anaplastic T-cell-lymphoma with cervical, retro-/supraclavicular, mediastinal, axillary and retroperitoneal lymphadenopathy as well as pulmonary manifestation. Two chemotherapy treatment lines and autologous stem cell transplantation resulted in a partial remission. To improve remission prior to HSCT the patient received 2 courses of brentuximab-vedotin. After conditioning therapy with fludarabine, busulfan, cyclophosphamide and ATG, HSCT from a HLA compatible unrelated donor was performed in April 2014. A PET-CT-scan in November confirmed complete remission. After HSCT the patient remained lymphocytopenic with cell count of CD4+ cells < 100/µl. After acute stage III gastrointestinal graft-versus-host disease (GvHD) low dose immunosuppressive therapy was maintained due to mild chronic GvHD of the liver and the upper gastrointestinal tract. Beginning in June 2015 the patient experienced increasing fatigue, general weakness, loss of appetite, nausea, night sweating and fever. Abdominal ultrasound, urine and blood culture as well as CT scans revealed no focus of infection. Different lines of empirical antibiotic therapy resulted only in short term improvement. Several blood culture tests remained sterile. A FDG-PET-CT scan showed a paraaortal and parailiacal lymphadenopathy with a high FDG uptake (SUV between 19.7 and 20.1), highly suggestive of lymphoma relapse. Endoscopic evaluations revealed two polypoid lesions in the bulbus duodeni. Histology of duodenal biopsies revealed a massive accumulation of weakly PAS-positive bacilli. PCR analysis confirmed an infection with mycobacterium genavense. Despite several attempts mycobacteria were not recoverable on solid media even by long term culture. Treatment was started with rifampicin, ethambutol, ciprofloxacin and clarithromycin. Lymph node manifestation responded to therapy with decreasing FDG-uptake (SUV 8.2) in a control FDG-PET-CT scan 3 months later. After 9 months treatment was terminated due to therapy refractory nausea. Lymphocytopenia was persisting with CD4+ cells < 100/µl. Six weeks after stopping the antibiotic therapy, symptoms as fever and weakness reappeared. Duodenal biopsy could not confirm persistent mycobacterial infection. FDG-positive intraabdominal lymph nodes (SUV 11.2) and spleen (SUV 8.2) were detected in a control FDG-PET-CT-scan. Five lymphnodes were surgically removed. Immunohistology detected histiocytic cell proliferation with no sign of lymphoma relapse. PCR confirmed the presence of mycobacteria-DNA. Consequently, antibiotic treatment was resumed. Mycobacterium genavense can present with all the symptoms of a lymphoma relapse and should be considered in immune compromised patients. Reliable diagnosis can only be obtained from lymph node biopsies and/or endoscopic evaluation. Treatment has to be accompanied by restoring cellular immunity and should only be stopped after PCR-negative biopsies.

**Disclosure of conflict of interest:** None.

**P287****Stratification of patients with multiple myeloma and lymphoma undergoing autologous hematopoietic stem cell transplantation in term of antifungal prophylaxis**

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Autologous hematopoietic stem cell transplantation (AHSCT) is at intermediate risk for invasive fungal infections (IFI). The recommendations of international scientific societies are not homologous regarding prophylaxis against IFI in patients (pts) undergoing AHSCT. The primary end point was to assess risk factors for the need of empiric/preemptive antifungal therapy in AHSCT recipients, and to extrapolate to the subgroup of pts that requires antifungal prophylaxis in our population of AHSCT pts. The secondary endpoint was to determine the fungal species distribution infecting or colonizing the pts. Our study included adult pts (> 18 yo) who underwent AHSCT for lymphoma and multiple myeloma (MM) between 2005 and 2015. All febrile neutropenic pts are being managed according to the 2010 Infectious Diseases Society of America (IDSA) guidelines regarding the use of antimicrobial agents in neutropenic pts with cancer. Eligible pts were divided into two groups: those who received empirical antifungal therapy and those who did not need it. We recorded demographic and baseline clinical characteristics including: age, gender, comorbidities, stage, disease status at AHSCT, high-dose therapy regimen, the presence of mucositis and its grade, the number of CD34 + cells transfused, the presence of central line or portacath, the need for mechanical ventilation, the presence of diarrhea, the duration of neutropenia, and the presence of bloodstream infections. Pts who had lung infiltrates suggestive of IFI were analyzed separately. The causative fungal pathogens and colonizers were analyzed. Univariate and multivariate analysis of potential risk factors to assess further significance was performed using SPSS. 190 patients were included. 106 pts (56%) had lymphoma and 84 pts (44%) had MM. The need of empiric antifungal therapy was statistically more significant in lymphoma than MM pts ( $P < 0.01$ ). The presence of mucositis grade  $\geq 3$  showed a statistical significance for the need of antifungal therapy ( $P = 0.02$ ). In the lymphoma group, remission status (PR vs CR) was not a significant factor for the need of empiric antifungal therapy ( $P = 0.49$ ). The presence of mucositis grade  $\geq 3$  was at the limit of significance ( $P = 0.05$ ). In the MM group, remission status (PR vs CR) did not affect the need of empiric antifungal therapy ( $P = 1$ ). However, mucositis grade  $\geq 3$  was found to be a significant risk factor for the need of empiric antifungal therapy ( $P = 0.02$ ). Following factors: the number of CD34 + cells transfused, the presence of central line and portacath, the need for mechanical ventilation, the presence of diarrhea, the duration of neutropenia, and bloodstream infections did not show any significance for the need of antifungal prophylaxis in both groups. All recovered fungal isolates ( $n = 14$ ) were not from deep seated tissues biopsies or blood, and were identified as *Candida albicans* in 7 with lymphoma, and in 7 with MM. They reflected the candida ecology in this pts series rather than deep seated fungal infections. We suggest to give antifungal prophylaxis to all lymphoma pts because of the higher need of empirical antifungal therapy, and give antifungal prophylaxis to MM pts having a predisposition for severe mucositis. Fluconazole is the antifungal of choice for prophylaxis since all the fungal isolates were *Candida albicans*.

**Keywords:** Autologous hematopoietic stem cell transplantation, antifungal prophylaxis.

**Disclosure of conflict of interest:** None.

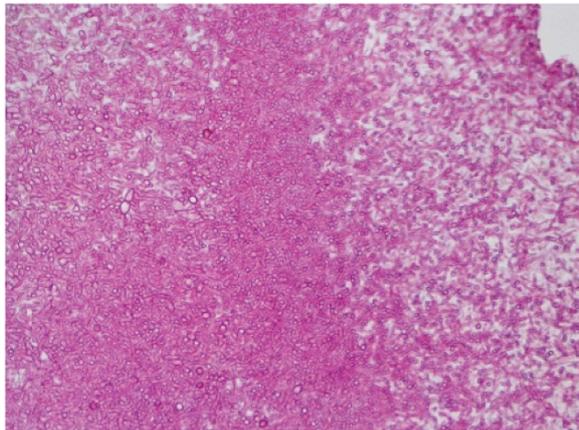
**P288****Successful management of CNS scedosporium infection after HSCT for aplastic anemia**

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A 48-year-old previously fit woman from a rural area of Eastern Europe was admitted to the hospital for severe aplastic anemia. Steroids, CSA, antifungal prophylaxis and supportive therapy were administered without response; therefore Rabbit ATG was then administered, with minor response; the year later, she underwent allogeneic-HSCT (MUD 9/10, RIC: TBI, Cyclophosphamide and Fludarabine; GVHD prophylaxis: ATG, CSA, MTX). Several days after transplantation she developed left migraine with ipsilateral back-eye pain. Brain MRI and CT showed a diffuse opacification of paranasal sinuses, mainly in the sphenoid sinus. The symptoms gradually improved with a specific treatment. The patient achieved a quick and complete haematological recovery and she was discharged. At follow-up visits she complained a flare of the migraine, with a left-sided headache that did not improve with NSAIDs. The headache gradually intensified until vision in the left eye became blurred with conjunctival injection. After consultation with Ophthalmologist, for suspected Toxoplasma retinitis, administration of intravitreal Steroids and Clindamicine was begun with partial benefit. However 15 days after (d +90) she was admitted in hospital because of worsening headache, irradiated in the occipital area, and weakness in the right hemibody. Tests on CSF were negative for neurotropic pathogens. An MRI showed a complete occlusion of the intracranial tract of left internal carotid artery, with likely infectious material localized in the left lateral cerebral fissure. A chest TC showed a nodule with initial excavation in the right superior pulmonary lobe. For suspected Tuberculosis she started antitubercular therapy. Despite a second lumbar puncture confirmed pleocytosis compatible with acute purulent meningitis, microbiological research for bacteria, fungi and BK were negative. So antitubercular and antitoxoplasma therapy were stopped and the patient underwent surgical biopsy within the sphenoid sinus. Pathological examination of the biopsy specimens showed acute and chronic inflammation of the respiratory mucosa, periodic acid. Schiff and Grocott staining (Figure 1) highlighted several septate fungal hyphae. Cultural analysis revealed colonies of *Scedosporium apiospermum* so the patient started targeted Voriconazole intravenous therapy. Nevertheless, 10 days later, she developed aphasia and right hemiparesis. A brain angi-MRI confirmed the appearance of new lesions compatible with infectious localizations associated to an increased defect of left internal carotid artery vascularisation and complete left choroid detachment. After 2 weeks of Voriconazole a significant clinical improvement have been observed and she was discharged, continuing oral antifungal therapy with Voriconazole. At the last follow-up she achieved a complete resolution of neurologic symptoms, with permanent left eye blindness. 8 months later (d +370) she was asymptomatic, with normal haematological and neurological conditions and was able to stop the antifungal therapy. This case-report confirms that the risk of invasive fungal infection (IFI) is relevant in patients receiving HSCT for AA, probably due to the prolonged neutropenia and association of other risk factors such as the immunosuppressive therapy and the iron overload. In this very poor prognosis infection, the early diagnosis of CNS IFI remains challenging, but the administration of Voriconazole was extremely effective.

[P288]



Disclosure of conflict of interest: None.

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Previously published

P290

**The effect of donor Epstein–Barr virus (EBV) seropositivity on graft versus host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (Allo-HSCT)**

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EBV seropositivity is detected in 80% of normal population. Reactivation of latent infection in post-transplant setting refers to EBV related posttransplant lymphoproliferative disease. The effect of donor EBV seropositivity on graft versus host disease is not well defined. In this study, we aim to present the seroprevalence of EBV and incidence of posttransplant lymphoproliferative disease as well as to evaluate the relation with GVHD. Between 2006 and 2015, the EBV serology of 364 patients that underwent allogeneic hematopoietic stem cell transplantation and their donors were evaluated in the study. EBV Ig G (VCA-IgG, EBNA ig G, EA-IgG) and IgM (VCA-IgM) antibodies were detected by chemoluminescence method (Abbott, ABD). All patients were followed for reactivation. EBV IgG seropositivity was detected in 338 patients (93%) and 238 donors (77.7%). There was no statistically difference in related vs unrelated transplants in seropositivity. The median age of the patients was 37 (range: 16–67), 217 patients were male (60%) and 295 (81%) had malign disease. The stem cell source was peripheral blood in 299 (82%) patients and 258 (71%) received grafts from related donors. Myeloablative conditioning regimen was received by 273 of patients (75%) (Table). All patients received acyclovir prophylaxis (related transplants 400mg TID, unrelated transplants 800 mg TID) during and after allo-HSCT up to 3 months. Twenty six-year-old pretransplant EBV seropositive aplastic anemia patient had EBV Ig M positivity after 3 months of allo-HSCT and developed lymphoproliferative disease. He was in complete remission after 4 courses of rituximab and methylprednisolone. Three patients were EBV IgM seropositive in 4th, 9th and 24th months of allo-HSCT and received symptomatic treatment. Acute GVHD was detected in 223 patients (61%) whereas 285 patients (78%) had chronic GVHD. Acute GVHD and chronic GVHD incidences were similar in comparison of donor EBV seropositive vs seronegative status (78% vs 22%,  $P=0.72$ ; 80% vs 20%,  $P=0.199$ ). EBV seropositivity was detected in 92.8% of patients. The donor EBV serology was not related with acute or chronic GVHD.

[P290]

Table. Patient Characteristics

Variable	Frequency n, (%)
<b>Sex</b>	
Male/Female	217 (60%)/147 (40%)
<b>Diagnosis</b>	
Malign Disease (most common acute leukemia)	295 (81%)
Benign Disease (most common aplastic anemia)	69 (19%)
<b>Donor</b>	
Full-mismatch Related	258 (71%)
Others	106 (39%)
<b>Stem Cell Source</b>	
Peripheral Blood	299 (82%)
Bone Marrow	65 (18%)
<b>Conditioning Regimen</b>	
Myeloablative	273 (75%)
Reduced Intensity	91 (25%)

Disclosure of conflict of interest: None.

P291

**The UMC Utrecht pediatric experience with brincidofovir after allo HSCT**

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Viral reactivation with DNA viruses form a considerable complication of allogeneic hematopoietic stem cell transplantation (HSCT). There are little effective antiviral therapies and most have considerable toxicity. Especially for Adenovirus, there is no satisfactory therapeutic option. Recently a new oral antiviral agent, the Cidofovir prodrug Brincidofovir became available to European patients only on the basis of urgent medical need and after a case by case approval by the health authorities. The aim was to describe our single center experience with Brincidofovir in the pediatric allogeneic HSCT setting. In the UMC Utrecht, pediatric patients receive T-replete bone marrow or Unrelated Cord Blood (UCB) as the donor source after mostly myeloablative conditioning regimens (+ serotherapy in unrelated-HCT). As GVHD prophylaxis patients receive Cyclosporine A (CsA) and MTX for bone marrow, CsA and prednisone for UCB. Patients are by standard weekly monitored for the presence of adenovirus, EBV, CMV en HHV6 viremia by RT PCRs in the plasma. Extensive immune reconstitution measurements are performed every 2 weeks. Since 2015, patients that developed viral reactivation with Adenovirus, or a combination of other DNA viruses (CMV, BK or HHV6) were offered Brincidofovir if the viremia was progressive or in the context of poor immune reconstitution. Brincidofovir was given in suspension (10 mg/ml) at the dose of 2 mg/kg BIW, or 100 mg BIW for larger children. The drug was discontinued when the viral load was below detection level. In total, six pediatric patients (age range: 0–18) received Brincidofovir (2 patients tablets, 4 the suspension). Four received it for Adenovirus reactivation, a 5th patient for CMV and BK and a 6th patient for CMV en HHV6. The median day post-HSCT of the first administration was 29 days Post HSCT (range: -4 to 101), the median day post detection of viral reactivation 14 days (7–76). The median duration of administration was 36 days (10–98) with two patients being discontinued because of death. In no patient the drug was discontinued due to toxicity issues. The patients that died had multi-organ failure due to a combination of severe aGVHD and multiple infectious issues. The patients were discontinued when the viral load was low and when they had CD4 counts of at least 50/ $\mu$ l. None of the four alive patients reactivated after the drug was discontinued. Urgent medical need

administration of Brincidofovir is feasible. In our limited series we found the drug was well tolerated.

**Disclosure of conflict of interest:** I am a medical consultant for Brincidofovir (Chimerix).

## P292

### Three cases of Herpes virus reactivation after allogeneic stem cell transplantation

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Reactivation of herpes simplex virus 1 (HSV-1) or varicella-zoster virus (VZV) occurs frequently after allogeneic stem cell transplantation (aSCT). Here, we report three unusual cases, two with reactivation of HSV-1 and one with VZV. Patients and methods: Patient (pt) 1 (50-year-old, male) was allografted for high risk acute lymphoblastic leukemia in first complete remission after conditioning with total body irradiation (12 Gy) and etoposide (60 mg/kg). Graft-versus-host disease (GVHD) prophylaxis was performed using cyclosporine A, short course methotrexate and anti T-lymphocyte globulin (ATG). Pts 2 (44-year-old, female) and 3 (67-year-old, male) were allografted for acute myeloid leukemia in second and first complete remission, respectively. Conditioning regimens used were FLAMSA-RIC in pt 2 and fludarabine/busulfan in pt 3. In both cases, GVHD prophylaxis consisted of cyclosporine A, mycophenolate mofetil, and ATG. Pts 1 and 2 had already experienced HSV-1-positive oral mucositis following induction chemotherapy and had successfully been treated with acyclovir. Both developed HSV-1-positive oral mucositis again after aSCT. In both cases, initial therapy with acyclovir i.v. at a dose of up to 10 mg/kg t.i.d. was ineffective. To explore the mechanism leading to clinical acyclovir resistance, the thymidine kinase genes of both viral strains were sequenced. Pt 3 presented with severe abdominal pain and nausea 11 months after aSCT. In this case, acyclovir prophylaxis post aSCT had been stopped 2 months before due to side effects. Moreover, low dose prednisolone therapy was necessary for chronic GvHD. The HSV-1-strain from pt 1 showed a single base pair deletion in the region from nucleotide position 430 to 436 of the thymidine kinase gene (which consists of a guanosine repeat). In pt 2 a single base pair insertion in the same region was found. Both genetic alterations lead to a loss of enzyme activity and acyclovir resistance. In both pts treatment was changed to foscarnet which led to rapid improvement. In the case of pt 3, multiple mucosal erosions were found on endoscopy of the esophagus. In these VZV DNA was detected by polymerase chain reaction (PCR). Only 4 days later, a vesicular skin eruption developed, which did not follow a dermatomal distribution. Again, in the vesicular fluid VZV DNA was detected by PCR. In this patient, acyclovir (10 mg/kg i.v., t.i.d.) resulted in rapid improvement. Reactivation of HSV-1 and VZV after aSCT is a frequent finding. Usually, HSV-1 strains respond well to acyclovir. In some cases, resistance can develop, especially in patients that had been treated with acyclovir before. Acyclovir resistance of HSV-1 caused by mutations in the thymidine kinase gene can be overcome by treatment with foscarnet which directly inhibits the viral DNA polymerase. Disseminated VZV reactivations after aSCT have been described. Clinical presentation can be misleading, for example, beginning with severe abdominal pain that precedes the vesicular eruption by several days.

**Disclosure of conflict of interest:** None.

## P293

### Toxoplasmosis disease in paediatric hematopoietic stem cell transplantation: do not forget it still exists

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Toxoplasmosis is a rare but severe complication after hematopoietic stem cell transplantation (HSCT) (1). It can involve the central nervous system alone or can manifest as a disseminated disease. In the paediatric population the mortality rate is high and sequelae are often severe. New diagnostic tools, such as the PCR assay, may allow for rapid diagnosis and preemptive therapy (2, 3). We retrospectively analysed all children who underwent allogeneic HSCT in our centre between January 2011 and December 2015. Patients lost to follow up before day +100 were excluded. Patients and donors were tested before transplant in order to assess their immunological status against *T. gondii*. A total of 187 allo-HSCT were analysed. Before transplant, 28.8% of recipients (R) were toxo-IgG positive and 71.2% were toxo-IgG negative. Among donors (D), serology was available only for 152/187: 23% were toxo-IgG positive, 77% were toxo-IgG negative. We found a high number of not tested donors (18.7%, 35/187) which included, in most cases, MUD from foreign registries. The group at higher risk for toxoplasmosis, D-/R+, included 21.7% pairs, whereas D-/R- were 55.2%, D+/R- were 15.1% and D+/R+ were 7.9%. In our series the cumulative incidence of toxoplasmosis disease was 2.1%, with 4 cases out of 187 transplants. Two of them (case 1 and 3) had cerebral toxoplasmosis, one (case 2) had disseminated toxoplasmosis and case 4 had toxoplasmic chorioretinitis. Mortality rate was 50%: two patients died because of multiorgan failure and disseminated toxoplasmosis respectively. In no case localized cerebral toxoplasmosis was the main cause of death. No complications were seen in surviving patients. All patients who developed toxoplasmosis were toxo-IgG positive before HSCT and three of them were transplanted from a toxoplasma IgG negative donor (fourth donor not tested). In the two fatal cases the Interferon-Gamma Releasing Assay (IGRA) never became positive, confirming the absence of specific cellular immunity. Toxoplasmosis disease can affect HSCT outcome in paediatric recipients and pre-HSCT seropositivity is the most important risk factor for toxoplasma disease in the post transplant period. In our cohort seroprevalence was higher than expected, probably due to the high number of patients coming from Eastern Europe. In order to reduce the burden of toxoplasmosis disease in our population we decided to implement a real-time PCR screening protocol for D-/R+ pairs, to provide rapid diagnosis and early therapy. All positive recipients with a seronegative donor will undergo real-time PCR screening starting on the day of stem cells infusion, and regularly until CD4+ T cell recovery. In the future we will analyse the impact of this strategy in this particular subset of immunocompromised patients.

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**Disclosure of conflict of interest:** None.

**P294****Treatment with brincidofovir for adenovirus disease in pediatric hematopoietic transplants**M González-Vicent<sup>1</sup>, B Molina<sup>1</sup> and MA Díaz<sup>1</sup><sup>1</sup>Stem Cell Transplant Unit, Hospital Niño Jesus, Madrid, Spain

Treatment with brincidofovir for adenovirus disease in pediatric hematopoietic transplants Introduction Adenovirus may cause serious morbidity and mortality after allogeneic hematopoietic transplants in children. Severe lymphopenia is the main risk factor associated with progression to disseminated and often fatal disease. Treatment with unlicensed cidofovir is based on monitoring of plasma viral load by PCR. However, cidofovir is only moderately effective at controlling adenovirus and it is associated with significant renal toxicity. Brincidofovir is a lipid conjugate of cidofovir. It has a good oral bioavailability and achieves higher intracellular levels of active drug than cidofovir with a better safety profile. It is a potent inhibitor of viral DNA synthesis so it could be indicated in immunocompromised patients with adenovirus disease. Patients and methods We present three children of 3, 5 and 9 years old diagnosed of acute lymphoblastic leukemia (ALL) in 2nd complete remission (the first two patients) and severe aplastic anemia the last one. There were 2 girls and 1 boy. They underwent a peripheral blood hematopoietic stem cell transplantation using  $\alpha\beta$ /CD19 depletion with a haploidentical donor in the two patients with ALL and CD45RA depletion with a matched unrelated donor in the other patient. Patients that underwent haploidentical transplants developed early acute graft versus host disease grade III with gut and skin involvement so immunosuppressive treatment with corticoids was started. They developed severe lymphopenia ( $<300/\text{mm}^3$ ). In the first month after transplant an adenovirus disease was diagnosed in the three patients from the weekly monitoring of plasma viral load by PCR. Adenovirus was also tested in stools, urine and respiratory sample. In all patients adenovirus was also detected in urine sample. In one of them adenovirus was detected in nasal exudate too and in the other the virus was isolated in stools and in a skin biopsy. **Results:** All of them were initially treated with cidofovir with poor results. Foscarnet and gancyclovir was also used without improvement. Finally they started a treatment by compassionate use with oral brincidofovir twice a week. With the first dose of brincidofovir plasma viral load started to go down until its complete disappearance. Brincidofovir tolerance was good [P295]

**Table 1 Patient Characteristics (n=28)**

Characteristic	Value
Age, median (range)	57.8 (38-72)
Lymphocyte count ( $\times 10^9$ ) at vaccination, median (range)	0.57 (0.02-2.98)
Days from transplant to vaccination, median (range)	78.5 (24-363)
Donor type, n(%) Sibling	8 (28.6)
Unrelated	20 (71.4)
Immunosuppressive therapy (IST) at vaccination, n (%)	18 (64.3)
GVHD at vaccination, n (%)	8 (28.6)
Intravenous Immunoglobulin in previous 12 months, n (%)	2 (7.1)
Anti CD20 monoclonal antibody in previous 12 months, n (%)	3 (10.7)

with only mild and limited diarrhea in two cases in the day they were taking brincidofovir. Two of the three patients were alive without signs of adenovirus disease. In the other patient blood adenovirus load by PCR decreased below 1000/mL, but remain high in urine. She died of respiratory failure due to pulmonary graft versus host disease. Conclusion Brincidofovir may be a promising therapeutic option for the treatment of severe adenovirus disease in immunocompromised patients with a good toxicity profile.

**Disclosure of conflict of interest:** None.**P295****Viral microneutralization assay to determine seasonal influenza vaccine immunogenicity in the first year post reduced intensity conditioning allogeneic HSCT**PDE Miller<sup>1</sup>, T de Silva, H Leonard<sup>2,3</sup>, K Goddard<sup>4</sup>, C Anthias<sup>3,1</sup>, K Hoschler, K Peggs<sup>5</sup>, A Madrigal and JA Snowden<sup>4,6</sup>

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International guidelines recommend that the seasonal inactivated influenza vaccine (IIV) is administered to allogeneic HSCT recipients. However, IIV immunogenicity studies using the Haemagglutination Inhibition (HI) assay have shown poor responses in the first year post-HSCT, reporting seroconversion rates (four-fold increase in titre) from 0–8%1. The Viral Microneutralization (MN) assay is highly sensitive, and can detect functional neutralizing antibodies below the threshold of HI2. Seroresponse to IIV by MN in HSCT recipients has not been previously described. The study was approved by NHS England Research Ethics Committee. 28 patients in the first year post-allogeneic HSCT were vaccinated, as part of standard care and in accordance with local HSCT programme policy, between October 2015 and February 2016 with a single dose of IIV containing 15  $\mu\text{g}$  Haemagglutinin (HA) each of A/California/7/2009 (H1N1)pdm09, A/Switzerland/9715293/2013 (H3N2) and B/Phuket/3073/2013. Baseline and 28-day post-vaccination antibody titres were determined by HI and MN. Patient characteristics are shown in Table 1. All patients were transplanted with PBSC for haematological malignancy, and

received reduced intensity conditioning (RIC) regimens with *in vivo* T-Cell depletion. The proportion of patients with baseline and post-vaccination HI titres  $\geq 1:40$  were 28.6 and 25% for A(H1N1)pdm09, 14.3% at both time points for A (H3N2), and 32.1 and 25% for B/Phuket. Pre and post-vaccination geometric mean titres GMT) were higher by MN than HI for A(H1N1)pdm09 and A(H3N2), but lower for B/Phuket ( $P=0.05$ ). No post-vaccination seroconversions were detected by HI, while a single seroconversion to A(H1N1)pdm09 was detected by MN in a patient vaccinated at 0–3 months. The MN assay did not detect any additional low-titre seroresponses (negative to detectable titre) below HI threshold. None of patient age, lymphocyte count, days from transplant to vaccination, donor type, and GVHD or IST at vaccination correlated with baseline or post-vaccination titres by either assay.

Response to IIV was virtually absent throughout the first year post-HSCT, with a single seroconversion to A(H1N1)pdm09 detected by MN but not HI, although the sample size was small and half of patients were vaccinated at 0–3 months. There is a clear need for a novel, immunogenic seasonal IIV and/or novel vaccination regimens in this population. Vaccination of recipients' relatives and close contacts, and HSCT healthcare workers should be strongly encouraged.

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**Disclosure of conflict of interest:** None.

#### P296

**Previously published**

## Early complications/late effects & quality of life

#### P297

### Symptomatic assessment of iron overload and confirmation of efficacy and safety of deferasirox in post-allo-HSCT setting

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Pre- and post-transplant iron overload (IO) has been associated with considerable long-term morbidity and mortality in pts undergoing transplantation. Classically, management of IO in the post-allo-HSCT setting has been based in the performance of therapeutic phlebotomies (TP), which are inconvenient for the patient and are often not feasible due to ongoing anemia. We recently published the first prospective study of deferasirox in adult allo-HSCT pts with IO (Vallejo, *et al.* *Haematologica* 2014). In this retrospective analysis, we analyzed the real-life management of IO in the post-allo-transplant setting. This study includes the last 113 pts with a minimum follow-up of 6 weeks, who underwent allo-HSCT in our center (October 2014–October 2016). 63 pts were male (55.8%) and 44 female (44.2%). Median age was 53 years (range: 7–69). Baseline diseases were: AML (44.2%), lymphoproliferative disorders (16.8%), MDS (12.4%), ALL (8.8%), chronic myeloproliferative diseases (7.1%), MM (5.3%), and BM failures (5.3%). Donor was unrelated in 61 cases (54%; 14 of them HLA mismatched), and related in 52 (46%; 21 of them haplo-identical). Conditioning regimen was: busulphan-based (68.1%), melphalan-based (13.3%), TBI-based (7.1%), and

others (11.5%). Progenitors source was PB in 102 (90.3%), and BM in 12 (9.7%). Pre-HSCT: pts had been transfused with a median of 23 PRBC (range: 0–147), and their median serum ferritin (SF) was 1359 ng/mL (range: 22–5116). Day +180 post-HSCT: 15 pts had died, and 24 pts had not reached that day yet, so 74 pts were evaluable. They had been transfused with a median of 31 PRBC (range: 0–157), and their median serum ferritin (SF) was 1127 ng/mL (range: 56–7993). 55% pts had SF superior to 1000 ng/mL. Liver MRI (by SIR method) to assess liver iron concentration (LIC) was performed in 44 pts at day +180. Seven pts (15.9%) had no IO (LIC 0–2 mg/g), 12 pts (27.3%) had moderate IO (LIC 2.1–4.4 mg/g), and 25 pts (56.8%) had severe IO (LIC superior to 4.5 mg/g). Median LIC was 4.66 mg/g (range: 0.6–11.34). Among the 29 cases with history of more than 20 PRBC transfused and SF higher than 1000 ng/mL at day +180, 28 (96.6%) were proved to have liver IO by MRI; the other pt had IO in spleen. 30 pts started some kind of therapy to treat the IO: 6 pts with severe IO initiated a TP program and 24 pts (6 out of 12 with moderate IO, and 18 out of 25 with severe IO) initiated chelation therapy with deferasirox. The drug was started at low dose (2.5–5 mg/kg/day), and was increased if tolerated up to a maximum of 20 mg/kg/day. Of note, the majority of pts were also taken a number of medications (immunosuppressants, statins, antimicrobials, etc). 3 of those 24 pts (12.5%) did not tolerate the drug, and were changed to TP. For more details, see the table. (1) The combination of the history of PRBC transfusions and serum ferritin levels was, in the majority of cases, enough to assess the IO in the post-allo-HSCT setting. (2) Liver MRI (by SIR method) helped to assess IO in doubtful cases. (3) Deferasirox, initiated at low doses and increased if tolerated, was safe and its use helped to avoid the need of therapeutic phlebotomies for the majority of patients. This study reproduces, in a real-life setting, our previous findings in a prospective clinical assay.

[P297]

	Deferasirox	Phlebotomies
Initiated	24	6
Changed to phlebotomies	3	--
Deferasirox (dose)	5-20 mg/kg/day*	--
Phlebotomies (amount/periodicity)	--	300-450 mL/1-3 weeks*
Deferasirox (duration of treatment)**	7 months (1-17)	--
Phlebotomies (duration of program)**	--	8,5 months (1-13)
Phlebotomies (number)**	--	13 (1-28)
Finished	4	2
Continue	17	4

\* Based on tolerance; \*\* Provisional

**Disclosure of conflict of interest:** None.

#### P298

### A case-control study of risk factors of primary graft failure with a focus on associated early-onset severe infections

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Graft failure (GF) is a rare but devastating event after allogeneic haematopoietic stem cell transplantation (AHSCT), exposing the recipient to disease relapse, drawbacks of marrow aplasia, infections and death. The aim of this study was to analyse the risk factors associated with graft failure after AHSCT, with a specific focus on early-onset severe infections (ESI). We conducted a retrospective, observational, single-centre, matched case-control (1:2) study among adult

AHSCT recipients transplanted at the haematology department of our institution between 2008 and 2015, with a subsequent follow-up of 12 months. Engraftment was assessed at day+42 post-AHSCT. GF cases were classified as primary GF (PGF), defined as failure to achieve donor-derived absolute neutrophil count (ANC)  $\geq 0.5 \times 10^9/L$  or lasting more than 3 consecutive days without evidence of disease relapse and early-secondary GF (ESGF), referring to the loss by day 42 post-AHSCT of a previously functioning graft associated without evidence of disease relapse. Each case was matched with two controls according to underlying haematological disease, HLA Matching, stem cell source, intensity of conditioning and temporal proximity of AHSCT. Demographics, haematological and graft characteristics as well as ESI report were retrieved. ESI were classified in invasive fungal infections, viral infections (CMV, EBV, HHV-6, other viruses), toxoplasmosis and severe sepsis of bacterial origin. During the study period, 598 AHSCT were performed at our center. Seventeen (3.1%) GF cases were identified, of which 15 PGF and 2 ESGF, and were matched with 34 controls. In the descriptive analysis, GF and control populations did not significantly differ when considering demographics, haematological characteristics and hematopoietic stem cell source. Regarding pretransplantation status and graft characteristics, only disease status (progressive disease) and cell dose (both CD34+ and CD3+ cells number/kg) were associated with graft failure. The proportion of patients with  $\geq 1$  ESI before day 42 was significantly higher in cases than in controls (11/17 vs 11/34,  $P=0.038$ ), with an overall number of ESI events of 19 and 12 among cases and controls, respectively. Five cases had  $\geq 2$  concurrent ESI. The median time from AHSCT to the first ESI event for GF cases was 17 days (interquartile range (IQR), 11–24) vs 15 (IQR, 8–34) days for controls ( $P=0.779$ ). In the GF setting, the most prevalent infections were Herpesviridae infections ( $n=7$  including HHV-6  $n=4$ , EBV  $n=2$ , CMV  $n=1$ ), probable IFI ( $n=4$ ), severe sepsis of documented bacterial origin ( $n=3$ ), toxoplasmosis ( $n=2$ ) among whom one patient developed haemophagocytic syndrome. When further analysing subsets of ESI using logistic regression, only toxoplasmosis was a significant risk factor for GF ( $P=0.018$ ). Death related to an infection was proven for 8 GF patients vs 5 control patients ( $P=0.012$ ). The overall survival probability at 12 months was significantly lower in the GF setting than in control patients (HR = 2.59 (95% CI 1.25 – 5.36),  $P=0.01$ ). The survival rates at 12 months were 35.3% and 57.7% for GF and control patients, respectively. At our center, graft failure is statistically associated with early-onset severe infections, and already known graft characteristics such as cell dose and disease status. However, our study would need more power to increase its significance.

**Disclosure of conflict of interest:** None.

## P299

### Abnormal cervical cytology and allogeneic stem cell transplantation

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Allogeneic stem cell transplantation (ASCT) is a curative option for hematological disorders, especially malignancies. In immunosuppressed women after ASCT, the progression from cervical dysplasia to invasive carcinoma is accelerated, and cervical cancer is likely a more aggressive disease. Therefore, follow-up protocols after ASCT should include regular gynecologic evaluation with Papanicolaou (Pap) smears. We retrospectively evaluated 32 Pap smears in 20 women who underwent ASCT and searched the risk factors for abnormal cervical cytology. The median age at transplantation was 44.5 years (range: 22–65 years). The most frequent indication for ASCT was leukemia (70%), and 85% of the patients received a

transplant from a sibling HLA-matched donor. Stem cell source was peripheral blood in all patients. Myeloablative conditioning regimen was used in 50% of patients. Cyclophosphamide, busulfan and fludarabine were used in 20 (100%), 18 (90%) and 10 (50%) patients, respectively. Acute graft versus host disease (GVHD) occurred in 7 patients (35%) and chronic GVHD in 4 patients (20%). Secondary cancer (1 breast cancer) was reported in only one patient at 40 months after ASCT. The follow-up time was 23 months (range: 3–104 months). After ASCT, benign and abnormal Pap smears were found in 12 (60%) and 8 (40%) women, respectively. The median time between ASCT and development of abnormal cytology was 2 months (range: 1–11 months). Four (20%) women had at least one smear with atypical squamous cells of unknown significance (ASC-US), one (5%) had a low-grade squamous intraepithelial lesion (LSIL), one (5%) had atypical squamous cells/high-grade lesion (ASC-H) and one (5%) had ASC-US and ASC-H. One (5%) patient had malign smear. Two patients with ASC-H showed high-grade atypia mimicking cancer but had a negative follow-up. Patient who had malign smear died because of aorta dissection. Cervical biopsy showed cervical intraepithelial neoplasia (CIN) I in 3 (15%) women who had ASC-US or ASC-H. One patient was HPV-positive. We did not find any relationship between cervical cytological abnormality and clinical factors. After ASCT, patients are high risk for abnormal cervical cytology and secondary gynecological cancer. Regular surveillance of patients is the most important factor for decreasing the risk of developing cervical and other secondary cancers. Gynecologic examinations and cervical cytological testing after ASCT allows early diagnosis and effective management of cervical abnormalities.

**Disclosure of conflict of interest:** None.

## P300

### Previously published

## P301

### Acute kidney injury after nonmyeloablative allogeneic stem cell transplantation for lymphoma

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Kidney dysfunction is a frequent complication of allogeneic stem cell transplantation (SCT) and contributes to the morbidity and mortality of the procedure. Incidence of severe acute kidney injury (AKI) in patients undergoing nonmyeloablative allogeneic SCT for malignant diseases ranges from 14 to 47%. Lymphoma patients are often heavily pretreated through both chemotherapy and autologous SCT and may be at increased risk of developing kidney injury. We performed a retrospective analysis of 108 consecutive patients with lymphoma undergoing nonmyeloablative allogeneic SCT between 2004 and 2016 (Table 1). Acute kidney injury (AKI) within 100 days of allogeneic SCT was diagnosed and staged according to RIFLE-criteria, and severe AKI was defined as RIFLE stage I–E (> doubling of creatinine or > 50% decrease of eGFR). Chronic kidney disease was defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m<sup>2</sup> 1 year after allogeneic SCT. We performed multivariate logistic regression to evaluate potential risk factors for severe AKI. Severe AKI developed in 75 patients (69.4%). Reduced overall survival was observed in these patients, although not statistically significant. No significant associations were seen with age at transplantation, baseline kidney function or prior autologous SCT. Severe AKI was associated with acute graft versus host disease (GVHD) (OR 2.8,  $P=0.026$ ) and the use of an unrelated donor (OR 2.8,  $P=0.025$ ). Chronic kidney disease was observed in 20 (18.5%) of patients alive after 1 year. We report a substantially higher incidence of severe AKI after nonmyeloablative allogeneic SCT for lymphoma than has been reported for other malignancies. Acute GVHD and unrelated donor stem cell

source were associated with severe AKI, while prior autologous SCT, age and baseline kidney function were not.

[P301]

**Table 1. Demographics at baseline**

Median age (range)	55 (16-68)
Sex male, n (%)	76 (70.4)
Unrelated donor, n (%)	63 (58.3)
Prior auto SCT, n (%)	74 (68.5)
Conditioning Flu/Cy, n (%)	102 (94.4)
eGFR, mean $\pm$ SD	93.3 $\pm$ 21

**Disclosure of conflict of interest:** None.

**P302**

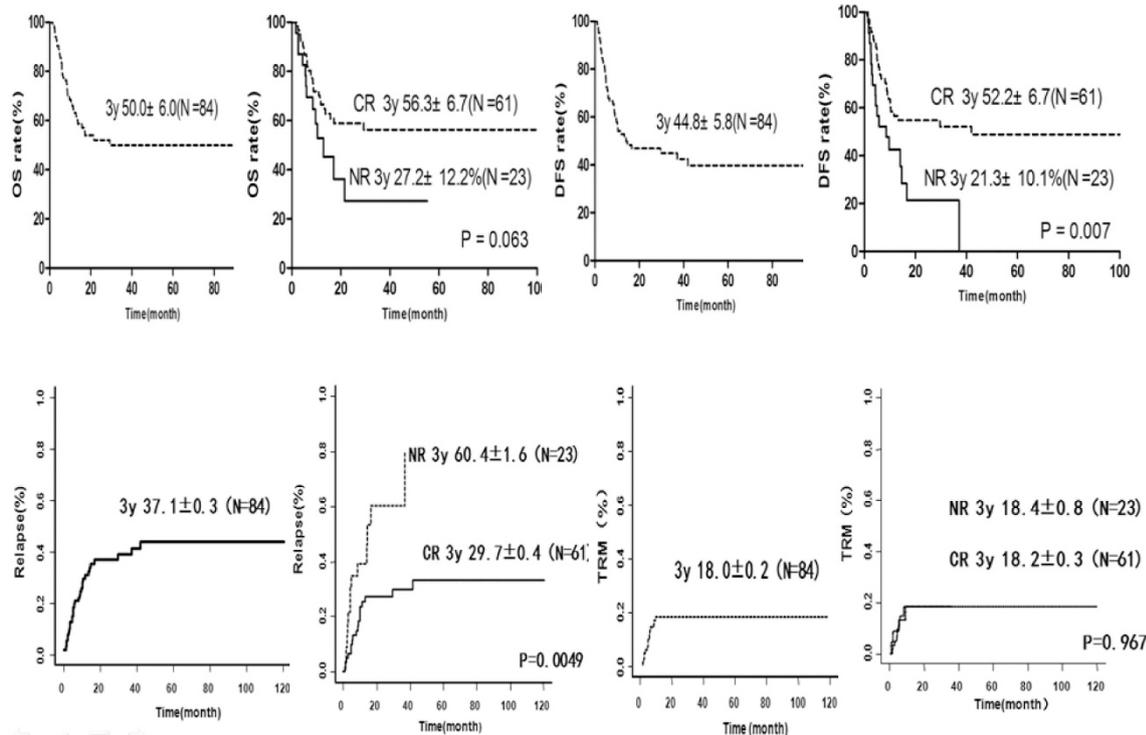
**Allogeneic hematopoietic stem cell transplantation for treatment of refractory and relapsed acute myeloid leukemia: long-term follow-up and prognostic factors**

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Patients with acute myeloid leukemia who are treated with conventional chemotherapy still have a substantial risk of relapse. We, therefore, retrospectively analyzed data to investigate the effects and some risk factors of allogeneic hematopoietic stem cell transplantation in relapsed and refractory acute myeloid leukemia patients, and to provide some suggestion for the clinical treatment. A total of 84

[P302]



refractory and relapsed acute myeloid leukemia patients receiving allogeneic hematopoietic stem cell transplantation in our center between February 2005 and December 2014 were retrospectively analyzed, including 23 patients in no-remission (NR) and 61 patients in second complete remission (CR2) at the time of transplant. The median age was 35 years (range: 9–55). Conditioning was myeloablative using cyclophosphamide, busulfan and total-body irradiation (Bu/Cy, n=44; TBI/Cy, n=3), and others were underwent nonmyeloablative stem cell transplantation. 81 patients had successful engraftment. Acute-GVHD and chronic-GVHD appeared in 47 and 37 patients. The 3-year overall survival (OS), relapse rate and disease-free survival (DFS) of the cases was 50  $\pm$  6.0%, 44.8  $\pm$  5.8% and 37.1  $\pm$  0.3%, respectively. The 3-year DFS were higher for patients in CR patients (52.2  $\pm$  6.7%) than in NR patients (21.3  $\pm$  10.1%), and the relapse rate in NR group and CR group were 60.4  $\pm$  1.6% and 29.7  $\pm$  0.4% respectively. There was no significant difference in treatment-related mortality compared CR group with NR group. Sex, age, related-donor graft were not independent factors affecting OS, DFS and relapse rate. It is concluded that allo-HSCT is an effective salvage therapy for patients with refractory and relapsed AML. Non-remission before transplant and severe aGVHD are high risk factors of poor prognosis for allo-HSCT. Patients in CR group who accept reinduction chemotherapy before transplantation have better prognosis than those in NR. The overall outcome seems related to the disease status. HSCT during refractory and relapsed can achieve long-term survival in selected patients with individual therapy.

**Disclosure of conflict of interest:** None.

**P303**

**Allogeneic hematopoietic stem cell transplantation (allo-HSCT) in advanced age adults: is it possible to transplant safely?**

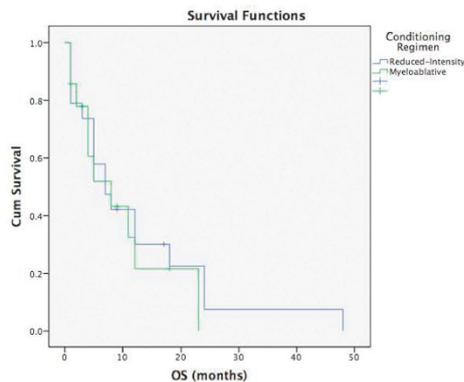
E Atilla, O Bas<sup>1</sup>, P Ataca Atilla<sup>2</sup>, S Civriz Bozdog<sup>2</sup>, M Kurt Yukse<sup>2</sup>, SK Toprak<sup>2</sup>, H Akan<sup>2</sup>, G Gurman<sup>2</sup>, M Ozcan<sup>2</sup>, M Beksac, O Arslan<sup>2</sup>, P Topcuoglu<sup>2</sup> and O Ilhan<sup>2</sup>

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**Table.** Patient Characteristics and Post-transplant Complications

Characteristics	Frequency (n,%)	PosttransplantComplications	Frequency (n,%)
<b>Diagnosis</b> AcuteLeukemia MyelodysplasticSyndrome Lymphoma CMPD-CMML MultipleMyeloma	16 (48%) 7 (21%) 5 (15%) 4 (12%) 1 (3%)	<b>AcuteGVHD</b> Grade II Gastrointestinal Grade III Gastrointestinal Grade I Skin Grade II Skin	4 (12%)  5 (15%) 1 (3%) 4 (12%)
<b>Stem Cell Source</b> Peripheral Blood Bone Marrow Cord Blood	30 (91%) 2 (6%) 1 (3%)	<b>Chronic GVHD</b> Gastrointestinal Skin Eye	8 (24%) 7 (21%) 1 (3%)
<b>Donor Type</b> Full Match Relative Donor Full Match Unrelative Donor 1 Mismatch Unrelative Donor Haploidentical-Cord	19 (58%) 5 (15%) 5 (15%) 4 (12%)	<b>Viral Infections</b> Sitomegalovirus (CMV) BK virüs Others (RSV, Rhinovirus, HSV, Coronavirus, Influenza)	14 (42%) 4 (12%) 4 (12%)
<b>Pretransplant Disease Status</b> Active Disease Remission	20 (61%) 13 (39%)	<b>Fungal Infections</b> Aspergillus Candida Mucor	11 (33%) 5 (15%) 1 (3%)
<b>Conditioning Regimen</b> Reduced Intensity Myeloablative	19 (58%) 14 (42%)	<b>Veno-occlusive Disease</b>	3 (9%)
<b>Graft vs Host Disease Prophylaxis</b> CSA+MTX CSA+MMF Tacrolimus+MMF	18 (55%) 11 (33%) 4 (12%)	<b>Thrombotic Thrombocytopenic Purpura</b>	3 (9%)
<b>Sorrer Score (HCT-CI)</b> 0 1 2	5 (15%) 24 (73%) 4 (12%)	<b>Hemorrhagic Cystitis</b>	4 (12%)

**Figure .** OS in patiens with reduced-intensity vs myeloablative conditioning



The incidence of most hematologic malignancies increases with age. Aging is related with a greater prevalence of impaired functional status and comorbidities. Although cure of malignant and non-malignant hematological diseases is

potentially possible with allo-HSCT, it could lead to significant transplant-related mortality. Decision making about referral to allo-HSCT in older adults is a challenging task. In this study we aim to present our geriatric allo-HSCTs. From 2007 to 2016, 33

patients (age<sup>3</sup>60) underwent allo-HSCT in our center included to this retrospective study. Pre-transplant status as well as posttransplant toxicities, complications and outcomes were determined. The age distribution of the group: 27 patients was aged <sup>3</sup>60 and < 65, 5 patients was aged >65 and < 70, 1 patient was 71 years old. The median age of donors was 49 (range: 21–73). The pre-transplant patients' characteristics are given in the table. Remission was achieved in twenty-three (70%) patients. Twenty-six patients (79%) had neutrophil engraftment (>0.5×10<sup>9</sup>/L) at a median day of 19 (range: 10–41) and platelet engraftment (20×10<sup>9</sup>/L) at a median day of 20 (range: 14–54). Post-transplant complications are detailed in the table. Acute graft vs host disease (GvHD) was occurred in 10 patients (31%) and chronic GVHD in 12 patients (36%). Eight patients (24%) were diagnosed with a relapse and 1 year relapse-free survival was 15%. The 1-year and 2-year OS were detected as 30% and 12%. The most common reason for mortality was sepsis. The 1-year OS was higher in patients who had reduced intensity conditioning regimen and remission status pre-transplant however they were not statistically significant (30% vs 21%, *P*=0.6; 31% vs 25%, *P*=0.9)(Figure). Since increasing number of older patients being diagnosed with hematologic malignancies, this trend of increasing number of allo-HSCT will continue. Tolerability and effectiveness are lesser, toxicity is higher in older adults. Although study population is relatively small, reduced-intensity conditioning and pre-transplant remission status may be related to better survival. Comprehensive geriatric assessment may be considered prior to allo-HSCT for global evaluation.

**Disclosure of conflict of interest:** None.

#### P304

##### **Allogeneic stem cell transplantation at-home program, experience and safety in hospital clinic of Barcelona**

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Allogeneic hematopoietic stem cell transplantation (ASCT) is a procedure with high morbidity and mortality (10–20%) requiring a complex hospital infrastructure. Improved support measures and development of homecare units has allowed that ASCT at-home programs may be possible. Our center has launched a pioneering program in our country in patients with ASCT to perform at home the following of aplasia, control of immunosuppressive therapy (IST) and intravenous support from the D+1 of ASCT until the engraftment and independent ambulatory patient. To evaluate the patient safety, we compared the group of patients at-home (ASCT-OP) with a cohort of ASCT 'in patient' with similar characteristics (ASCT-IP). 26 ASCT patients between January 2014 and October 2016 at the Hospital Clinic of Barcelona. 13 patients performed ASCT-OP and 13 had an ASCT-IP. All patients received conditioning (myeloablative–MAC–or reduce intensity–RIC–) in the hospital with fludarabine 40 mg/m<sup>2</sup> (D1–4) and busulphan 3.2 mg/kg (2–4 doses), prophylaxis of GvHD was performed with tacrolimus/mycophenolate (MMF) in ASCT-OP group and cyclosporine(CsA) and methotrexate (MTX) or MMF in ASCT-IP group. In all patients, the infectious prophylaxis was conventional (levofloxacin, fluconazole and acyclovir). Moreover, the ASCT-OP group received prophylaxis with ceftriaxone 1 g intravenous (IV) once daily and liposomal amphotericin B inhaled 25 mg twice a week during neutropenia. The ASCT-OP group from D+1 received a nurse visit once daily and physician visits twice a week in the hospital. Baseline characteristics were analyzed those related to toxicity and patient outcomes. The median age (range) was 56 years (23–69), male/female 16/10; (62% male). The source of the progenitors was peripheral blood in all cases and analysis of the results detailed in the table:

#### [P304]

N=26	ASCT-OP (N=13) N (%)	ASCT-IP (N=13) N (%)
Age, median (range)	56 (48-69)	56 (23-66)
Gender		
Male	9 (69%)	6 (54%)
Donor		
Unreated	11 (85%)	8 (62%)
CD34+x10 <sup>6</sup> /Kg		
Median (range)	5.8 (2.3-8)	6.2 (2-8)
Diagnostic		
AML	9 (70)	9 (70)
MDS	2 (15)	2 (15)
Myeloproliferative diseases	1 (8)	1 (8)
Lymphoma	1 (8)	0 (0)
Multiple myeloma	0 (0)	1 (0)
Conditioning		
MAC	5 (39)	5 (39)
RIC	8 (61)	8 (61)
GvHD prophylaxis*		
CsA/MTX	0 (0)	8 (61)
CsA or TK/MMF	10 (100)	5 (39)
Days of follow-up/admission, median (range)	26 (20-31)	26 (21-38)
Days of neutropenia <0.5x10 <sup>9</sup> /L, median (range)	7 (6-21)	14 (6-23)
Days of platelets* <20x10 <sup>9</sup> /L, median (range)	2 (0-5)	6 (0-27)
Neutropenic fever*	3 (23%)	13 (100%)
Days of neutropenic fever* median (range)	1 (0-1)	3 (2-6)
Mucositis*		
Grade 3-4	1 (8)	5 (39)
GvHD +30d*	0 (0)	4 (31)
TRM +100d	0 (0)	0 (0)
* <i>p</i> <0.05		

The ASCT, MAC or RIC underwent at home is a safe procedure. There seems to be a trend in lower incidence of neutropenic fever and early GvHD in the ASCT-OP group.

**Disclosure of conflict of interest:** None.

#### P305

##### **An increase in RDW-SD after allogeneic hematopoietic transplantation is associated with a poor prognosis**

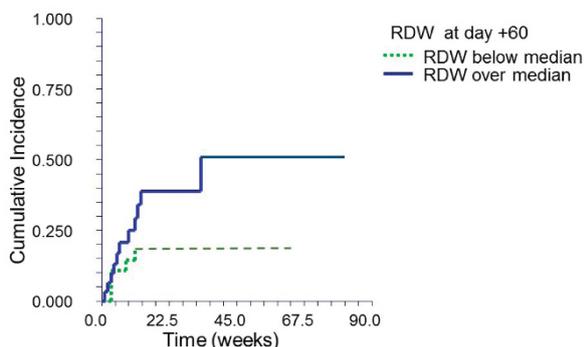
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Red cell distribution width (RDW), is an erythrocyte index influenced by stress erythropoiesis, inflammation and antioxidants. RDW predict mortality in sepsis, chronic kidney diseases and in cardiovascular disease. No data are available on RDW after hematopoietic transplantation. In a retrospective study we collected data on changes of RDW-SD in a group of 81 patients who received allogeneic hematopoietic transplantation. Forty-eight patients were affected by Acute Leukemia, 13 by lymphoma, 9 by MM, and 11 by other diagnosis. RDW was studied at baseline and monthly for the first 3 months. A subset of 34 patients were studied prospectively for clinical and laboratory signs of microangiopathy. At baseline before the transplant a RDW-SD higher than normal upper limit was observed in 43% of allogeneic candidates. A high co-morbidity score (HTC-CI score 2–5) at the pre-transplant screening was a factor associated to high RDW-SD ( $\chi^2$  *P*=0.01). A value of RDW-SD higher than normal range, at baseline, was not associated to any other factors, such as age, diagnosis, phase of the disease, previous transplantation, C-reactive protein, bilirubin, creatinine and arterial hypertension. Early after allogeneic transplant we noticed at day +30 a significant reduction of RDW-SD but subsequently (at day +60) the proportion of patients showing an abnormal RDW-SD increased to 63%. An abnormal RDW-SD at

day +60 was registered in 70% of allogeneic transplant patients who presented an acute GVHD while in only 30% of patients who did not presented during the first 3 months an acute GVHD ( $\chi^2$   $P=0.02$ ). In allogeneic transplantation group, patient who, at day +60, had a RDW-SD higher than normal value had a inferior outcome in respect to patients having a RDW-SD within normal ranges (OS was 70% vs 30%; logrank:  $P=0.04$ ), (CI of TRM: 45% vs 18%). These two groups were not significantly different for pre-transplant features. In the subset of patients studied prospectively, abnormal RDW-SD was associated to presence of schistocytes in PB (chi test: 0.004) and patients having  $\geq 2\%$  schistocytes had a median RDW-SD of 71 (IQR 31) vs a median RDW-SD of 46 (IQR 12.2) in patients who did not show schistocytes in PB (Mann-Whitney  $U$ -test  $P=0.004$ ). RDW-SD was significantly correlated also to serum triglycerides ( $r=+0.4$ ,  $P=0.0004$ ) and to red blood cell mean corpuscular volume ( $r=+0.32$ ,  $P=0.02$ ). Abnormal RDW-SD is frequent after allogeneic transplantation. Abnormal RDW-SD is associated to acute GVHD and its value obtained at day +60 marks a group of patients with poor prognosis because of high TRM. This simple parameter warrant further studies to determine its clinical usefulness in monitoring of patients suffering acute-GVHD and in diagnosis and monitoring transplant associated microangiopathy.

[P305]

TRM ACCORDING TO RDW MEASURED AT +60



**Disclosure of conflict of interest:** None.

### P306

#### Assessment of impact of hematopoietic stem cell transplant on sickle cell disease burden index

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Sickle cell disease (SCD) poses a lot of psychological burden for the patient and the caregiver. It also poses a significant financial burden over the family. Ohaeri *et al.* developed a 16 point questionnaire to assess sickle cell disease burden called as sickle cell disease burden index (SCDBI) and its impact on caregiver's quality of life (QOL). We used this questionnaire to assess the impact of hematopoietic stem cell transplant (HSCT) on caregiver's QOL. 16 point questionnaire was sent to 15 set of parents whose child underwent HSCT between January 2016 and June 2016. SCDBI contained 16 questions in various domains (3:family finances, 3:family interactions, 5:routine family activity and 5:parental coping ability). Answers were graded on a score of 0–3 (0:never occurred and 3:occurred regularly or had a severe impact on the family). The results were interpreted in two headings A. family finances and interactions (0: no impact; 1–3: insignificant impact; 4–6: moderate impact; 7–9: severe impact) and B. routine family activity and parental coping ability (0: no impact; 1–5: insignificant impact; 6–10: moderate impact; 11–15: severe impact). All these domains were assessed before and after HSCT. Ten parents replied with duly filled questionnaire. Mean age at HSCT was 8.1 years (range: 1–14), M/F:7/3. All were symptomatic for >6 months before HSCT with 90% having more than 2 hospital admissions.

Majority of parents were from middle class with median family income of 30 000 USD per annum (range 16 000–200 000 USD). Median score for family finances and interactions (A) before HSCT was 6 (range: 1–19) which decreased to 0 (range: 0–3) after HSCT. Median score for routine family activities and parental coping ability (B) before HSCT was 13 (range: 3–25) which decreased to 0 (range: 0–6) after HSCT. Our results suggest that before HSCT there was a moderate impact on family finances and interactions which reduced to no impact after HSCT. Similarly there was severe impact on family activities and parental coping ability before HSCT which changed to no impact after HSCT. Our study suggests that HSCT not only improves the QOL of the child but also of the caregivers.

#### Reference

1. Ohaeri JU, Shokunbi WA, Psychological burden of sickle cell disease on caregivers in a Nigerian setting. *J Natl Med Assoc* 2002; **94**: 1058–1070.

**Disclosure of conflict of interest:** None.

### P307

#### Previously published

### P308

#### Autologous serum preparations for treatment of ocular lesions in chronic graft versus host disease: a 12-year single-centre experience

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Chronic graft versus host disease (cGVHD) is a late complication of allogeneic hematopoietic stem cell transplantation (HSCT) that affects many tissues and organs and manifests with polymorphic clinical features similar to autoimmune diseases. Poorly understood pathophysiological mechanisms are implicated in inflammation and tissue fibrosis which is a hallmark of cGVHD. The affection of lachrymal glands is frequent and contributes to ocular manifestations presenting as dry eye syndrome. Autologous serum eye drops (AESDs) are used topically to facilitate tissue healing and ease the symptoms in a variety of ocular diagnosis. It is unclear if the serum of a patient with cGVHD is suitable for remedy preparation and if the transplanted patient himself can meet the criteria for autologous donation. Aim is to show the safety, feasibility and efficacy of autologous serum preparations in ocular lesions after allogeneic HSCT. Donors should meet criteria for autologous blood donation (infectious disease status, complete blood count Hgb > 110 g/L, Hct > 33%, adequate venous access). AESDs are prepared from 150 ml of autologous blood left to clot, irradiated and centrifuged to separate serum which is diluted with saline in 1:5 ratio or 1:1 if requested. Product is dispensed into 1.5 ml ampules, stored at -20 °C and a 3-month supply is released to the patient after receiving negative results of sterility testing. In period from 2005 to 2016. in the AESDs program 12 patients (4 female, 8 male) with ocular symptoms were included. All met required predonation criteria. Of 29 collections performed, one failed due to venous access problem and one product had to be discarded due to hemolysis. cGVHD global NIH score of the patients at start of the program was: 4 severe, 6 moderate, 1 mild and 1 not scored. All patients presented with moderate to severe dry eye symptoms. In 6 (50%) patients AESDs alleviated dry eye symptoms. In 3 (25%) out of 12 patients referred to AESD program, more than 3 autologous blood collections were performed (range: 4–10) and AESDs were used regularly through period of 10–36 months, which points to the beneficial effect of the long-term use of the serum. Three patients dropped out because AESDs showed no advantage compared to commercial lubricant eye drops preparations. One patient dropped out because of a venous access problem, 4 patients had disease

progression and needed other therapies: 3 cases of amniotic membrane application of which 2 continued with AESDs to facilitate the healing effect. One patient was recently included and the effect of AESD is still evaluated. Autologous donations in cGVHD patients are feasible, safe and autologous serum preparations can help relieve symptoms of dry eyes. It needs to be further elucidated specifically in which patients and at what point of the disease course the effect of the AESDs is the most beneficial to make optimal use of these preparations.

**Disclosure of conflict of interest:** None.

### P309

#### Biomarkers for idiopathic pneumonia syndrome after hematopoietic cell transplantation: predictors for occurrence and survival

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Idiopathic pneumonia syndrome (IPS) is a non-infectious pulmonary complication with diffuse lung injury that develops in 5–10% of patients who undergo hematopoietic cell transplantation (HCT) and the mortality rate remains high at 80%. The major aim of this study was to identify prognostic biomarkers for IPS and establish positive and negative predictive values (PPV and NPV) of IPS. In a case–control study, we compared 41 patients with IPS with available samples (transplanted between 1988 and 2014 at FHCRC) with 162 HCT control recipients who did not require bronchoscopic examination and who did not grow any bacterial or fungal blood cultures. For each subject, plasma samples at day 7 post HCT and onset of IPS or matched time points for controls were analyzed. The ‘onset sample’ for controls was the sample closest to day 24 (median day of onset for patients with IPS). We measured six proteins by ELISA: Suppressor of tumorigenicity 2 (ST2), tumor necrosis factor receptor 1 (TNFR1), interleukin-6 (IL-6), lymphocyte vessel endothelial receptor (LYVE)-1, endothelial protein C receptor (EPCR), and herpes virus entry mediator (HVEM). Multivariable logistic regression models were used to evaluate the association of each protein with IPS vs controls. Cytokine cutoff values that maximized discrimination between IPS and controls were identified using Receiver Operating Characteristic (ROC) analysis. PPV and NPV of IPS were calculated using the identified cytokine cutoffs across a range of hypothetical IPS prevalence values (0–15%). Day 200 weighted Kaplan–Meier survival curves were estimated for high/low cytokine subgroups. Similarly, a weighted log-rank test was used to evaluate *P*-values. A multivariable logistic regression model including six cytokines showed that ST2 and IL-6 were significantly important markers to identify IPS at the onset (Table 1). ST2 value at day 7 post HCT was significantly associated with occurrence of IPS and IL-6 had a marginal association. Predictive values for IPS by a plausible percentage of the actual HCT population (up to 15%) are shown in Figure 1. Of the six proteins, ST2 showed the highest PPV both at onset and day 7 post HCT followed by TNFR1, and IL-6. NPV were high in all the markers. To analyze whether ST2 and IL-6 at day 7 after HCT can predict survival following IPS, we dichotomized the patients into cytokine high and low groups (cutoff level: ST2, 19 ng/mL; IL-6, 35 pg/mL) and compared survival after down-weighting the observations to represent a plausible percentage of the actual population (IPS prevalence, 5%). Day 200 survival rate were significantly lower in ST2 high value group than in ST2 low value group (80% vs 88%, *P*=0.015). Similarly, IL-6 high value was associated with high mortality (day 200 survival rate,

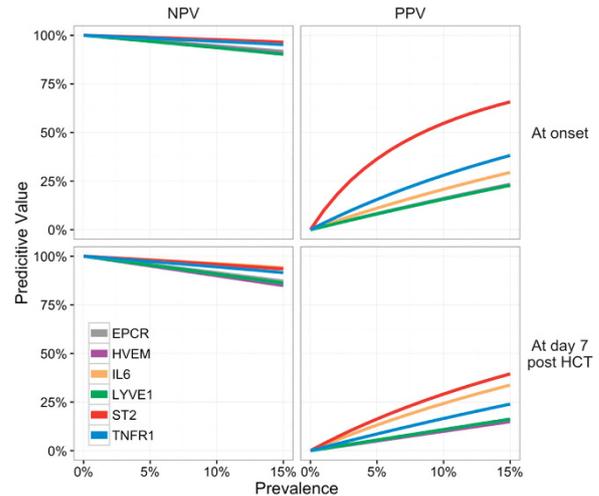
78% vs 88%, *P*=0.003). ST2, IL-6, and TNFR1 were good prognostic markers for occurrence IPS. Especially, ST2 and IL-6 at day 7 after HCT can be a predictor for both IPS occurrence and survival following IPS. These results require validation in an independent prospective HCT population.

#### Reference

1. Seo *et al. Blood* 2015; **125**: 3789–3797.

[P309]

Multivariable models predicting IPS				
Sample collection time	Cytokine	Odds ratio	95% CI	p-value
Onset	ST2	2.8	2.0-4.0	<0.001
	IL-6	1.4	1.0-1.9	0.025
Day 7 post HCT	ST2	2.0	1.5-2.8	<0.001
	IL-6	1.3	1.0-1.6	0.06



**Disclosure of conflict of interest:** SP has a patent on biomarkers licensed to Viracor-IBT laboratories.

### P310

#### Body composition changes in stem cell transplantation: the case of lymphoma patients

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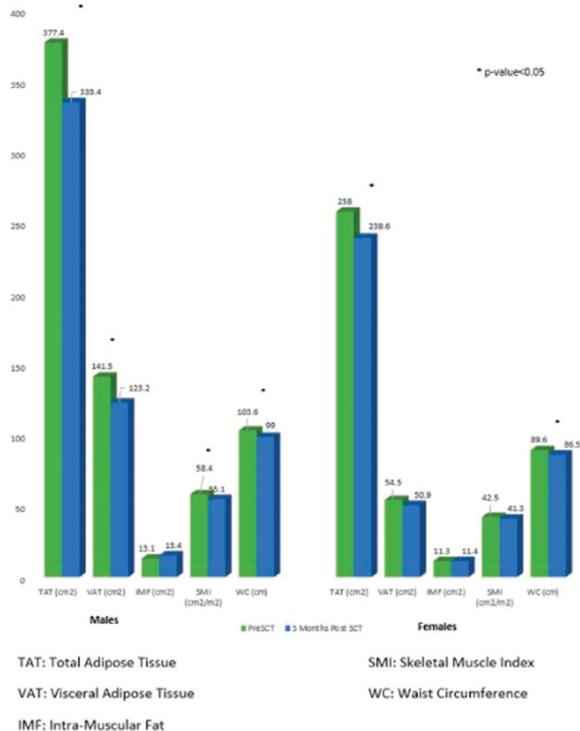
Body composition parameters are sensitive nutritional indicators that influence response to treatment and mortality in cancer patients. Research is not conclusive on the changes in muscle attenuation and adipose tissue areas in the stem cell transplantation (SCT) phases. Objective is to assess the changes in adipose tissues, skeletal muscle index (SMI) and waist circumference (WC) among stem cell recipients in the peri-transplantation phase. **Study Design:** Institutional Review Board approved this retrospective study with 61 adult patients (age > 16 years) having B and T lymphoma who underwent SCT. Each patient was imaged by PET/CT scan pre-SCT and 3 months post transplantation. A cross sectional image was analyzed at the level of the L3 to calculate Total Adipose Tissue (TAT), Visceral Adipose Tissue (VAT), Intra-Muscular Fat (IMF), SMI and WC. Data was analyzed by

gender since body composition parameters differed significantly between the two categories in the literature. The study sample consisted of 61 patients (mean age: 38.2 ± 13.7 years, 35 (57%) males, 51(83.6%) autologous SCT, median overall survival in months: 39.8 in males and 40.5 in females). Death was observed in 6 (17.1%) males and 1(3.8%) female. Patient characteristics were similar for males and females except for weights (kg) and body mass index (kg/m<sup>2</sup>): 86.7 and 28.6 vs 63.5 and 24.1 in males and females respectively. Changes from pre-SCT to 3 months post SCT revealed that TAT, VAT, SMI and WC decreased with mean differences of 42 ± 61.2 cm<sup>2</sup>, 18.28 ± 37.6, 3.3 ± 7.5 cm<sup>2</sup>/m<sup>2</sup> and 4.58 ± 5.4 cm, respectively in males (*P* < 0.01). In females, TAT and WC significantly decreased with mean differences of 18.4 ± 37 cm<sup>2</sup> and 3.1 ± 4.3 cm, respectively (*P* < 0.01). In females, VAT and SMI decreased clinically but did not reach clinical significance. In multivariate analysis, no significant associations were shown with mortality and progression rates. This study fills a research gap by providing data on the evolution of body composition parameters in the peri-transplantation phase. TAT, VAT, SMI and WC decrease 3 months post transplantation. Future studies should evaluate the associations of these parameters with major outcomes on larger sample sizes.

[P310]

Table 1. Patient Characteristics

Variable	Males (n=35)	Females (n=26)
Age at transplantation, mean ± s.d.	40.3 ± 14.2	35.4 ± 12.7
Disease, n (%)	Hodgkin	17 (48.6)
	Non Hodgkin	18 (51.4)
Type of transplantation, n (%)	Autologous	29 (82.9)
	Allogeneic	6 (17.1)
Conditioning Intensity, n (%)	Myeloablative Conditioning	34 (97.1)
	Reduced Intensity Conditioning	1 (2.9)
BMI at admission (Kg/m <sup>2</sup> ), mean ± s.d. *	28.6 ± 5.6	24.1 ± 5
Overall Survival, median (range), months	39.8 (10-297)	40.5 (12-147)
Alive, n (%)	29 (82.9)	25 (96.2)



Disclosure of conflict of interest: None.

### P311

#### Bone complications following hematopoietic cell transplantation

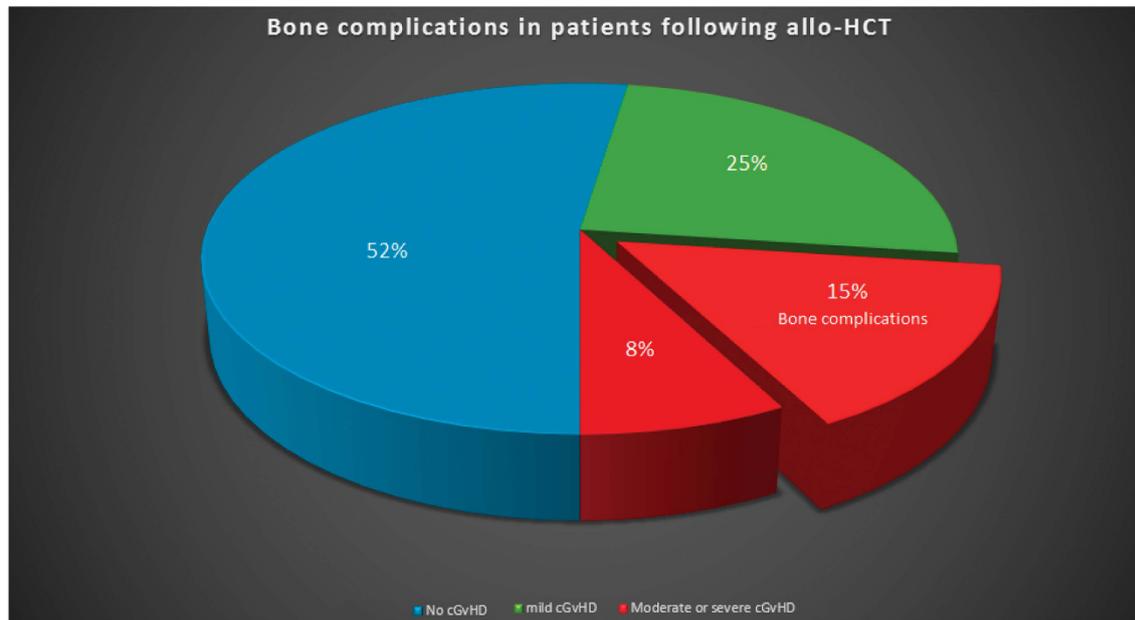
E Karakulska-Prystupiak, G Basak, J Dwilewicz-Trojaczek and WW Jędrzejczak  
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According to literature data, HCT survivors are at increased risk for bone complications which may significantly affect their activities of daily living and quality of life. The aim of this study was to assess the frequency and risk factors of bone complications in patients transplanted with allo-HCT. A retrospective analysis involved a group of 105 patients of the Outpatient Transplantation Service of the Department of Hematology, Medical University of Warsaw transplanted in the years 2010–2016 and evaluated for the years 2014–2016. 78 patients received myeloablative conditioning (MAC) and 27 reduced intensity conditioning (RIC). Fifty patients suffered from chronic graft versus host disease (cGvHD) including 24 with grade moderate or severe. Magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry scan (DXA) were used to assess of bone complications. The bone mineral density (BMD) was measured according to the WHO scale. In addition, baseline measurements of endocrine and serum levels of 25-hydroxy-vitamin D were performed in all patients. Bone complications were diagnosed in 18 patients (9 AML, 4 ALL, 1MDS, 1AA, 1CML, 2 HD) (aged 15–56 years, median age: 37 years): 7 women and 11 men, within 1–4 years (median: 2 years) post allo-HCT (6-RIC, 12-MAC). 5 patients received TBI-containing preparative regimen. All these patients were exposed to calcineurin inhibitors for prevention and treatment of GvHD. 16 patients suffered from cGvHD-grade moderate or severe. All patients required systemic corticosteroids, because of GvHD (16pts) or during basic treatment of lymphoma (2 pts). All patients had deficient states of vitamin D initially and required replacement. All of them, except for 2 patients, had balanced adrenal insufficiencies and 2 patients had balanced hypothyroidism. All women had premature ovarian failure (2 received HRT). According to measurements of bone mineral density (BMD), low bone mass was detected in 15 patients; osteopenia (11pts), osteoporosis (4pts). Bone loss of femoral neck (8-osteopenia, 3-osteoporosis) occurred more often than lumbar vertebral ( 6-osteopenia, 2-osteoporosis) or radius (3-osteopenia, 1-osteoporosis). Presence of avascular necrosis of bone (AVN), confirmed by MRI, was detected in 12 patients and the most common site of involvement was the femoral head(all patients), knee(3 pts) and shoulder(1 pts). One of the first symptoms of AVN was pain and functional limitation. All patients required intensive analgesic treatment, usually NSAIDs and 4 patients — fentanyl. Fractures occurred in 12 patients. The femoral neck (7 pts) and thoracic or lumbar vertebral (3 pts) were two most common fracture sites. All patients were qualified for surgery; 6 patients required hip replacement, 6 – patients still awaited to perform surgery or were disqualified because of severe, skin cGvHD. Bone complications may occur in about 17% of allo-HCT survivors (including 30% patients with GvHD, and up to 60% patients with severe or moderate cGvHD) within first 4 years after allotransplantation. Bone loss, particularly at the femoral head, is the most common complication. Avascular necrosis usually requires surgical intervention because of fractures. Exposure to higher doses of corticosteroids (during treatment of GvHD) increases risk of bone complications. Early diagnosis by MRI and DXA may help to detect bone complications.

#### References

Kwong Y et al. *Hong Kong Med J* 2008; 14: S17-S20.

Disclosure of conflict of interest: None.



**P312**

**Changes in intensive care for stem cell transplant patients: the need for response criteria at 5 days of full treatment to separate good risk patients and avoid futile interventions**

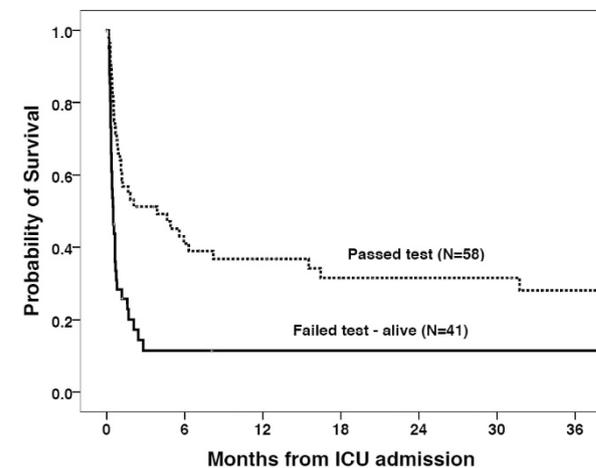
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Intensive care unit (ICU) admission is associated with high mortality in stem cell transplant (SCT) patients. Patients and methods: we retrospectively reviewed all onco-haematology patients admitted to the ICU between 10 October and 15 December. We classified pts according to the reason for ICU admission in 5 groups: (a) respiratory failure without mechanical ventilation during the first 24 h; (b) respiratory failure and mechanical ventilation in the first 24 h; (c) sepsis without respiratory failure and without renal replacement therapy in the first 24 h; (d) renal replacement therapy without respiratory failure regardless of septic status; and (e) needing hemodynamic support without respiratory failure, sepsis or renal replacement therapy in the first 24 h. After 5 days of full intensive therapy we defined a successful 5-day ICU trial for each of the five groups as follows: (a) no mechanical ventilation during 5 days; (b) neutrophils > 1.0 or ≤ 2 organ failures by day 5; (c) C-reactive protein decreased by 50% or normalised lactate by day 5; (d) off renal replacement therapy by day 5; and (e) no inotropic support on day 5. Patients who were discharged during the first 5 days of ICU admission were considered successes. 166 pts, with 202 ICU admissions. The median number of ICU admissions was 1 (1–4), with 138 (84%) having 1 admission, 20 (12%) 2 admissions, 4 (2.4%) had 3 admissions and 3 (2%) 4 admissions respectively. The median length of stay in ICU was 6 days (1–95). Disease status was complete remission (n = 77, 38%), partial remission (n = 28, 14%) and stable disease (n = 96, 48%). 30% of allogeneic SCT had MAC and 70% RIC and 29 (35%) were from HLA-identical sibling, 47 (58%) unrelated and 6 (7%) haploidentical donors. The reason for admission to ICU was respiratory failure for 107 (53%), sepsis in 39 (19%), renal failure in 32 (16%) and hemodynamic failure in 22 (11%). Overall 101(50%) pts survived their ICU admission and were discharged to the hematology ward. Of these, 31 (30%) died in hospital and 70 (70%) were discharged home. Estimated overall

survival was 15% (95% CI 10–23) at 3 years post ICU admission. For the assessment of parameters predictive of survival after 5 days on ICU, we selected 138 pts with one admission. The distribution according to the groups was: (a) 56; (b) 34; (c) 17; (d) 17 and (e) 14. Overall 58 pts (42%) reached our criteria for a successful ICU trial: (a) 30 (53%); (b) 14 (41%); (c) 4 (23%); (d) 7 (41%) and (e) 3 (21%). A total of 41 (30%) pts failed to meet these criteria but remained alive on day 5 and 39 (28%) died before day 5. The overall survival (Figure 1) for the 58 pts was 28% at 3 years with an overall mortality in ICU of 33% (19/58) compared to 71% (29/41) for those who did not meet our criteria. The overall survival for pts that met our criteria at fifth day and were discharged to the haematology ward (n = 39), was 49% at 3 years. In this study, 50% of patients survived their ICU admission. Patients could be stratified according to the reason for admission and given an individualized 5-day trial: those who met our criteria for successful ICU trial (42%) had a low ICU mortality (33%) and those who were subsequently discharged home had an overall survival of 49% at 3 years. This study raises the possibility of offering a short-term ICU stay to onco-hematologic patients and perhaps allows for the ceiling of intensive care for those who fail these criteria.

[P312]



Disclosure of conflict of interest: None.

**P313****Chronic GvHD as an independent risk factor for thromboembolic complications after allogeneic stem cell transplantation**

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For many patients suffering from hematologic malignancies allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment option. Unfortunately up to 80% of patients develop a chronic graft versus host disease (cGVHD) after allogeneic HSCT. Other frequent long term complications are cardio-vascular morbidities such as heart failure, coronary heart disease and thromboembolic complications (up to 23% after HSCT, Savani *et al*, *Blood* 2010). Mouse experiments showed that endothelial damage takes place after bone marrow transplantation and that there might be an association between GVHD and vascular changes. Inflammation as part of the deregulated immune system in GVHD could promote those vascular changes and increase the risk of cardiovascular complications. Here we analyzed if chronic GVHD and its inflammation are associated with thromboembolic complications in our patients after allogeneic HSCT. We retrospectively analyzed 414 patients with hematologic malignancies after allogeneic HSCT, transplanted between 2009 and 2014 at the University Medical Center Mainz. All patients were seen in an outpatient routine work up life long and got a GVHD screening according to the NIH consensus guidelines every 3 months (median follow up 3.8 years, range: 0.8–6.7 years). Our patient cohort consists of 247 male and 167 female patients with a median age of 52.8 years (range: 18.1–75.4). 50% suffered from AML (11.1% ALL, 10.1% lymphoma, 8.2% myeloproliferative disorder, 7.5% MDS, 7.2% multiple myeloma). 62.1% of patients received a dose reduced intensity conditioning (RIC) regimen, 27.3% a myeloablative chemotherapy, 10.6% with refractory acute leukemia were treated with a FLAMSA-RIC regimen. Donors were in 76.8% fully matched (10/10) unrelated donors, stem cell source mainly was peripheral blood stem cells. 112 patients (27%, 64 male and 48 female) developed a chronic GVHD. 59 patients showed a deep sclerotic phenotype. 80 (25%) of 318 patients with a HLA-identical transplant developed a cGVHD. In comparison, 32 (33%) of 96 patients with HLA-mismatched donor suffered from cGVHD. 4.6% of all transplanted patients developed a new thromboembolic complication after HSCT. 8.9% of the patients with cGVHD developed a thromboembolic complication, mainly deep vein thrombosis, some with pulmonary embolism, 2 patients had additional thromboembolic risk factors. In contrast, only 2.98% of patients without cGVHD showed a thromboembolic complication in the later time course, with one patient showing an additional thrombotic risk factor. In multivariate analysis cGVHD was an independent risk factor for thromboembolic complications after HSCT. 1.8% of patients with thrombosis before HSCT showed one afterwards. Thrombosis before HSCT was not found as risk factor for thromboembolic complication after HSCT. Our retrospective analysis showed an increased risk for thromboembolic complications after allogeneic HSCT, with substantial higher risk in patients with chronic GVHD (8.9%). In ongoing studies we currently investigate a vascular screening procedure with additional biomarkers according to inflammation and endothelial damage in patients with cGVHD prospectively. We hope to identify patients at risk for thromboembolism and prevent future complications on an individualized basis.

**Disclosure of conflict of interest:** None.

**P314****Previously published****P315****Clinical presentations and management of isolated extramedullary relapses after allogeneic hematopoietic stem cell transplantation for acute myelogenous leukemia: an updated single-institute analysis of 378 patients**

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Isolated extramedullary (EM) relapses of acute myelogenous leukemia (AML) are rare events, although increasingly have been reported as a significant contributor to post-transplant mortality following allogeneic hematopoietic stem cell transplantations (alloHSCT). We retrospectively analyzed incidence, clinical characteristics, treatment options and long-term outcome of this pattern of leukemia recurrence in a cohort of 378 consecutive patients (pts) who underwent alloHSCT in our center between June 1993 and March 2013. 53 pts relapsed (any site). 11 (21 %) out of all pts who relapsed (F/M 3/8, median age 40 years, range: 25–60 years) experienced histologically proven isolated EM relapse after a median time of 10 months (mts) (range: 6–80 mts) following allo-HSCT. 3 pts developed skin and/or subcutaneous tissue infiltrates. Other sites of relapse included (no. of cases): central nervous system (CNS) (3), paraspinal soft tissues (1), small intestine (1), lymph nodes (1), paranasal sinuses (1), breast (1). Treatment plans for those isolated EM relapses included (No. of cases): 1/ systemic chemotherapy (5), 2/ systemic chemotherapy and secondary alloHSCT (4), 3/ imatinib, then dasatinib (1), 4/ surgery (1). Pts with CNS involvement received intrathecal therapy with cytarabine and in one case additional CNS irradiation was applied. 10/11 pts died after a median time of 9 mts (range: 1–25 mts) due to resistant systemic relapse, infectious complications or extensive graft-versus-host disease following alloHSCT. 1 patient remains alive and disease-free at 88+ mts following secondary alloHSCT. Conclusions: Our data indicate that EM disease following alloHSCT affects a significant proportion of pts with AML. Sites of EM relapses vary widely among the pts with skin and CNS being frequently involved. An aggressive approach combined of local and systemic therapy including secondary alloHSCT may produce favorable response in a small proportion of pts, however, overall prognosis for pts with isolated EM relapses still remains poor. Due to the lack of effective treatment strategies, there is a need for novel approaches to manage isolated EM relapses after alloHSCT.

**Disclosure of conflict of interest:** None.

**P316****Previously published****P317****Comparison of various diagnostic criteria of thrombotic microangiopathy after stem cell transplantation**

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Hematopoietic stem cell transplantation (HSCT)-associated thrombotic microangiopathy (TMA) is a multifactorial complication, and has variable incidence in study populations due to different diagnostic criteria. **Aim:** Our aim was to identify pediatric patients with HSCT associated TMA using 4 different diagnostic TMA criteria published in literature and to compare the various groups for TMA parameters and outcomes. We enrolled 33 pediatric patients who underwent allogeneic HSCT using treosulfan based or reduced intensity conditioning therapy. 4 different TMA diagnostic criteria, the BMT CTN

Toxicity Committee Consensus Definition (1), the Overall Thrombotic Microangiopathy Grouping (2), the diagnostic criteria created by City of Hope (3) and the criteria proposed by Jodele *et al.* (4) were used to stratify the patients. We determined and registered the following TMA activity markers: presence or development of increased LDH and decreased haptoglobin levels, new onset anemia, thrombocytopenia, fragmentocytes, Coombs test, kidney function, proteinuria, hypertension and terminal complement complex (sC5b-9). Complement pathway activities, components and sC5b-9 were measured during early HSCT period. Two/33 (1), 7/33 (2), 3/33 (3) and 10/33 (4) subjects met the different TMA diagnostic criteria according to the four different systems on day 12 and 34 (1) and on median 44 (2), 43 (3), 61 (4) post-HSCT days. All of the 6/10 patients who were defined with the first three criteria, met the fourth definition. Due to normal haptoglobin levels and kidney function, 4/10 patients fulfilled only the fourth criteria. TMA coexisted with acute graft-versus-host disease in 7/10 cases (7/10 vs 4/23;  $P < 0.01$ ). Patients who met any of the different TMA diagnostic criteria had higher sC5b-9 level on day 28 (411 vs 201 ng/ml;  $P = 0.003$ ) compared to those without. All of the 10/33 subjects defined with TMA had elevated sC5b-9 ( $> 250$  ng/ml) level during the early HSCT period. Two patients died before day 100 after HSCT, out of which one patient met all of the four TMA diagnostic criteria. After a median 2.29 (1.2–3.1) year follow-up time, overall survival was 24/33. 8/10 patients with TMA survived, compared to 16/23 patients without TMA. Relapse related mortality was the most common cause of death ( $N = 7/9$ ,  $P < 0.05$ ), while TMA was not a significant cause of mortality after reduced toxicity conditioning therapy. HSCT-associated TMA has a variable and complex pathophysiology. Using the different diagnostic criteria may influence the incidence and the time of diagnosis of this transplant-related complication. Monitoring all of the published TMA activity parameters, including complement terminal pathway activation marker, may help to guide physicians to recognise TMA after HSCT.

**Disclosure of conflict of interest:** None.

**P318**

**A comparison of the predictive value of the HCT-CI index and the EBMT score to assess the overall survival, relapse incidence and non-relapse mortality in hematopoietic stem cell transplantation: a single center experience**

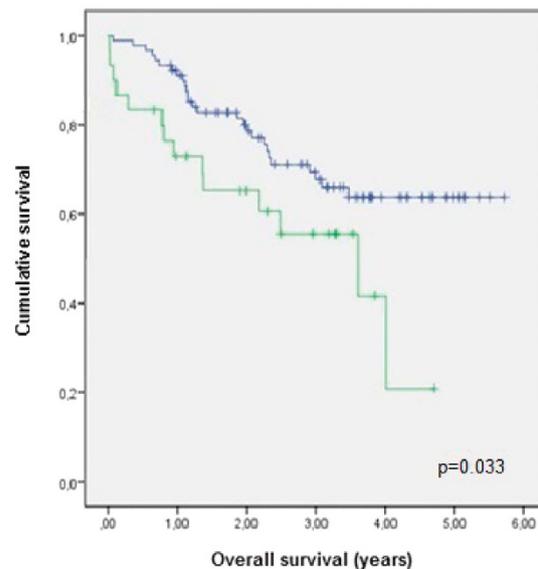
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Hematopoietic stem cell transplantation (HSCT) is associated with a risk of non-relapse mortality (NRM). It's important to assess the risk of complications and mortality before the HSCT. Some indexes quantify the impact of patients' comorbidities on HSCT outcome. The most frequency used is the HCT Comorbidity Index (HCT-CI) and the European Group for Blood and Marrow Transplantation score (EBMTs). This study tried to determine which of the two indexes best predicts the outcome in a series of patients submitted to HSCT in a single center. Between 2011 and 2015, 259 HSCT were performed in our center. A total of 215 HSCT have been analyzed (we excluded patients  $< 18$  years (yr), 2<sup>nd</sup> HSCT, haploidentical donors and HSCT for specific diseases with very low number ( $< 3\%$ ) of HSCT performed: aplastic anemia, CLL, prolymphocytic leukemia, mycosis fungoides, Sezary syndrome, dendritic cell neoplasia, plasma cell leukemia and POEMS syndrome). The HCT-CI and EBMTs were calculated retrospectively (yr 2011–2013) and prospectively (yr 2014–2015). Overall survival (OS), relapse incidence (RI) and NRM were analyzed in the overall series and separately according to the type of HSCT: autologous HSCT (auto-HSCT) or allogeneic HSCT (allo-HSCT).

Male: 89 (59%) patients. Median age: 54 yr (range: 18–71). Diseases: AML 54 (25%), ALL 19 (9%), MM 67 (31%), NHL 41 (19%), HL 12 (5%), MDS 17 (8%), CMPD 5 (3%). Disease status: 1<sup>st</sup> complete remission (CR) or 1<sup>st</sup> chronic phase 102 (48%),  $\geq$  second CR 30 (14%), first partial remission (PR) 55 (26%),  $\geq$  second PR 12 (5%), no response 11 (5%) and without previous treatment 5 (2%). Auto-HSCT in 120 patients (56%) and allo-HSCT in 95 (44%) patients. Related and unrelated donor were 52 (55%) and 43 (45%), respectively. The conditioning regimen was standard in 57 (60%) cases and reduced intensity in 38 (40%). HCT-CI and EBMTs grouped 0–2, 3 and  $\geq 4$  were 57 (48%), 29 (24%), 34 (28%) and 38 (32%), 51 (42%), 31 (26%) in auto-HSCT and 61 (64%), 18 (19%), 16 (17%) and 34 (36%), 32 (34%), 29 (30%) in allo-HSCT, respectively. Median follow-up was 3.15 yr (0.66; 5.73) for the overall series, 3.18 yr (0.66; 5.73) for auto-HSCT and 2.60 yr (0.97; 5.70) for allo-HSCT. Significant differences in OS and NRM were found according to the EBMTs in patients submitted to auto-HSCT. One-yr-OS and 3-yr-OS were 91% (95% CI: 85%; 97%) and 68% (95% CI: 57%; 79%), respectively, in patients with EBMTs 0–3, vs 73% (95% CI: 57%; 87%) and 56% (95% CI: 37%; 75%), respectively, in patients with EBMTs  $\geq 4$  ( $P = 0.033$ ). One-yr-NRM and 3-yr-NRM were 2% (95% CI: 0%; 7%) in patients with EBMTs 0–3, vs 13% (95% CI: 4%; 28%) in patients with EBMTs  $\geq 4$  ( $P = 0.015$ ). No significant differences were observed for RI according to EBMTs in patients submitted to auto-HSCT. No significant differences in OS, RI and NRM were observed according to EBMTs in patients submitted to allo-HSCT. No significant differences regarding OS, RI or NRM were found when the HCT-CI was assessed. In our series, only the EBMTs was predictive of OS and NRM in patients submitted to auto-HSCT. Failure to find statistically significant differences for the HCT-CI and for EBMTs in allo-HSCT recipients could be due to an insufficient number of patients or to a partial retrospective collection of data.

[P318]

**Figure 1. Overall survival in auto-HSCT based on EBMT score**



**EBMT score 0-3 (blue line):**  
 1-yr OS probability 91% (95%CI: 85%-97%)  
 3-yr OS probability 68% (95%CI: 57%-79%)

**EBMT score  $\geq 4$  (green line):**  
 1-yr OS probability 73% (95%CI: 57%-87%)  
 3-yr OS probability 56% (95%CI: 37%-75%)

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### P319

#### Conception and pregnancy outcomes after haemopoietic stem cell transplant: a retrospective study from the quality of life and complications working party

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Infertility is common after HCT predominantly as a result of the chemoradiotherapy used in conditioning. Nonetheless, some patients do retain or recover fertility. Newer reduced intensity regimens may be less gonadotoxic. In addition, patients are increasingly encouraged to store gametes, or embryos before transplant. We sent questionnaires to 602 EBMT centers requesting retrospective details of number of pregnancies and pregnancy outcome for all patients treated between 1995–2015. 27 centers responded from 13 countries detailing 234 patients who became pregnant/partners conceived. The most frequent underlying diagnoses were acquired bone marrow failure ( $n=44$ , 25F) AML ( $n=42$ , 15F), HD ( $n=26$ , 15F), CML ( $n=25$ , 7F), ALL ( $n=19$ , 4F) and B NHL ( $n=18$ , 10F). Other diagnoses included MDS, MPS, solid tumours, autoimmune disease, CLL, T-NHL, haemoglobinopathy. Of 110 females (F), 35 (32%) involved assisted reproductive techniques (ART). 30F had TBI (seven < 4 Gy) of which 16 (50%) had ART. 25F had reduced intensity conditioning of whom 6 (24%) had ART. 70F were specified as having standard conditioning of whom 24 (34%) had ART. 73F had allogeneic (26 ART, 36%) and 37F had autologous transplants (9 ART, 24%). Of 124 men (M) whose partners conceived, 61 (49%) had ART. 54M received TBI of which 36 (67%) had ART. Where specified, 19 had reduced intensity HCT (3 ART, 16%) and 94 had standard conditioning (48 ART, 51%). 93 had had allogeneic HCT (43 ART) and 31 autologous (12 ART). 19 men had reduced intensity transplants. 53 men received TBI (two < 4 Gy) of whom 36 (68%) had ART compared to 69 men without TBI, 16 (23%) of whom had ART. Data on return of menstruation was available for 84. 64 indicated yes and 12 (19%) had ART. 20 indicated amenorrhoea of whom 14 (70%) had ART. 224 specifying number of children had 324 live births (LB) and 87 (39%) patients had more than one child after HCT. 146 LB occurred in female patients (41 ART, 28%) and 178 LB were in partners of male patients (88 ART, 49%). The median gestational age for 61 female patients was 39 weeks (range: 22–42) and the median birth weight was 3 kg (range: 0.3–4.19). There were 3/80 congenital anomalies. The median follow up of the offspring was 5 y (range: 0–15). Developmental problems were indicated for 1/71 (fine motor skills) and learning difficulties in 1/70 (ADHD). In partners of male patients the median gestational age for 62 offspring was 39 weeks (range: 26–43). The median birth weight for 56 offspring was 3 kg (range: 0.87–4.16). Congenital malformations occurred in 4/89. One infant died of pulmonary infection. In women, several methods of assisted conception were used including hormone

stimulation, IVF, cryopreserved embryos, donor embryos and cryopreserved ovarian tissue. The most frequent method was use of donor embryos (22/35) in which a minimum of 30 attempts led to 21 LB. The median number of attempts was 1 (range: 1–5). ART were frequently used in this group of post-transplant patients particularly in male patients vs female, TBI vs non-TBI, amenorrhoeic vs menstruating women, standard conditioning vs RIC. In patients who conceive after HCT, successful pregnancy leading to healthy offspring is the likely outcome.

**Disclosure of conflict of interest:** None.

### P320

#### Danaparoid reduces the transplant related mortality for stem cell transplantation in children

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In stem cell transplantation (SCT), death from transplant-related complications is one of the major obstacles hindering improvement of transplant outcomes. Introduction of proper supportive care could reduce the transplant-related mortality (TRM) and improve the overall survival (OS). As for the transplant related complications, coagulopathy with or without microangiopathy often underlies organ damages, and correction of coagulopathy is essential for the prevention or treatment of transplant related complications. Since reports of the reduction of TRM that focused on the contribution of supportive care are very limited, OS and TRM in the past decades in our institution were analyzed to identify the contribution of supportive care, especially anticoagulants, to the reduction of TRM in SCT for children. A total of 210 pediatric patients with malignant ( $n=176$ ) and non-malignant ( $n=34$ ) disorders who consecutively underwent SCT in our institution from 2000 to 2013 were analyzed. As for the supportive care for the prevention of SOS, oral UDCA was given to all patients, and oral tocopherol acetate was given to 197 patients. In addition, intravenous dalteparin was given to 96 patients until September 2005, and then switched to danaparoid from October 2005 for 114 patients. Patients and transplant characteristics. SCT was either autologous ( $n=51$ ) or allogeneic ( $n=159$ ), and all SCTs for solid tumors were autologous grafts ( $n=40$ ). Stem cell sources were bone marrow (BM,  $n=125$ ), peripheral blood (PB,  $n=37$ ), and cord blood (CB,  $n=48$ ). Disease status at SCT in malignant disorders was complete remission (CR,  $n=118$ ) or non-CR ( $n=58$ ). The conditioning regimen was either myeloablative conditioning (MAC,  $n=161$ ) or reduced intensity conditioning (RIC,  $n=49$ ). The transplant years were divided into three periods, A (2000–2004), B (2005–2008) and C (2009–2013), and an improvement of 5-year OS and a decrease of 5-year TRM were observed over these periods of time, that is, OS was 61.5%, 60.3% and 79.5%, ( $P=0.062$ ), and TRM was 19.9%, 7.9% and 0.0%, ( $P<0.001$ ) in periods A, B and C, respectively. On multivariate analysis, non-malignant disorders (HR=0.165, 95% CI=0.040–0.674,  $P=0.012$ ), RIC regimen (HR=0.354, 95% CI=0.162–0.773,  $P=0.009$ ), and danaparoid (HR=0.609, 95% CI=0.384–0.966,  $P=0.035$ ) were independent prognostic factors of OS for all patients and disease status at SCT (HR=4.447, 95% CI=2.548–7.763,  $P<0.001$ ) for patients with hematological malignancies in allogeneic SCT. The prognostic factor for TRM for all patients was administration of danaparoid (hazard ratio (HR)=0.109, 95% confidence interval (CI)=0.033–0.363,  $P<0.001$ ), and in allogeneic SCT for patients with hematological malignancies, danaparoid (HR=0.046, 95% CI=0.006–0.326,  $P=0.002$ ) and advanced disease at SCT (HR=4.802, 95% CI=1.734–13.30,

$P=0.003$ ) were the independent prognostic factor of TRM. Reduction of TRM after SCT was observed over the transplant periods and supportive care with danaparoid was found to be significantly effective to reduce TRM. Therefore, prophylactic administration of danaparoid is considered to be a reasonable option to improve the transplant outcomes for children. [P320]

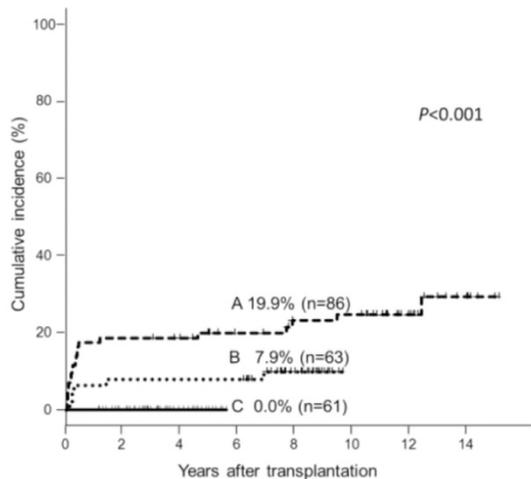


Figure 1. Five-year transplant-related mortality of all patients in period A (2000-2004), B (2005-2008), and C (2009-2013).

**Disclosure of conflict of interest:** None.

### P321

#### Deferasirox restores hematopoiesis in poor graft patients after allogeneic hematopoietic stem cell transplantation

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The attainment of transfusion independence after transplant is sometimes hampered by a combination of factors, ranging from infections to the need of combined therapy for clinical complications, as well as control of GVHD. Iron overload is frequently observed in hematological patients before and after hematopoietic stem cell transplantation (HSCT). Whereas several reports have focused on iron overload before transplant, up to now, this is the only report that show full recovery of hematopoiesis and correlate this to deferasirox chelation performed on this particular subset of patients. We report on 19 patients, transplanted for hematological diseases (17 acute leukemia, 1 aplastic anemia, 1 multiple myeloma) heavily transfused before transplant that, considering the iron overload, were treated with deferasirox after HSCT. Before starting deferasirox, the patients were fully engrafted and in complete remission, although transfusion dependent, and with incomplete hematological reconstitution after allogeneic HSCT. Patients were selected according to the following inclusion criteria: (1) transfused pre-transplant with more than 20 RBC units; (2) incomplete hematological recovery; (3) transfusion-dependence; (4) serum ferritin > 1000 ng/mL; (5) normal creatinine value. The workup for other aetiologies resulted negative. All patients received an initial dose of deferasirox 10 mg/kg/day, later adjusted according to side effects. All patients experienced an increase in hemoglobin levels, with a reduction in the frequency of RBC transfusions, followed by transfusion independence (median time: 24 days from the first dose of deferasirox). In addition, it was promptly (median time: 27 days) associated with hematological improvement, with sustained values and no further platelet

support or growth factors administration. No relevant modifications with immunosuppressive or myelosuppressive drugs were made during deferasirox treatment. Deferasirox was well tolerated. Basing on our results, we think that deferasirox determined stimulatory, and/or depressive effects on hematopoiesis after allo-HSCT. This clinical experience raises the possibility of a potential additive benefit on hematopoiesis after transplant following iron chelation therapy with oral deferasirox. Further long term studies, in larger cohorts of patients are needed to confirm these data and to design an efficient strategy to reduce iron loading after transplant.

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### P322

Previously published

### P323

#### Delayed onset subacute atypical hemolytic uremic syndrome (aHUS) post allogeneic stem cell transplantation (SCT)

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Thrombotic microangiopathy (TMA) is a well-described complication that can occur post allogeneic SCT. TMA typically occurs within the first 1–2 months post SCT, with an acute onset, often associated with significant morbidity and mortality. However, a subtype of TMA can occur with a delayed onset, after 3 months post SCT. TMA comprises several clinical entities that include thrombotic thrombocytopenic purpura (TTP) associated with a low level of ADAMTS13, typical Shiga toxin associated hemolytic uremic syndrome (HUS), and other subtypes of HUS which include idiopathic HUS, secondary HUS, and complement-associated TMA or atypical HUS. Between January 2013 and October 2016, five patients were diagnosed with late onset subacute atypical HUS post SCT at our institution. They were 3 males and 2 females aged 16–25 years. Diagnosis included acute lymphoblastic leukemia ( $N=3$ ), Hodgkin lymphoma ( $N=1$ ), immunodeficiency (hypomorphic Artemis mutation) ( $N=1$ ). Patients received a total body irradiation (TBI) based cytoreduction ( $N=2$ ) or a chemotherapy based cytoreduction ( $N=3$ ). Four patients received T-cell depleted grafts while one patient received an unmodified graft with tacrolimus and sirolimus for GvHD prophylaxis. Donors were matched siblings ( $N=3$ ) or unrelated ( $N=2$ ). Two patients developed grade 2–3 GvHD. Only one patient was on a calcineurin inhibitor. These five patients had recovered normal or near normal hematologic function. At 3–8 months post SCT patients developed (1) Coombs negative hemolytic anemia ( $N=5$ ) with minimum hemoglobin 6.3–8.6 g/L, with maximum LDH 410–950 U/L and undetectable haptoglobin, (2) thrombocytopenia with minimum platelets of  $9000\text{--}71000 \times 10^3/\text{mL}$ , (3) renal insufficiency ( $N=4$ ) with maximum creatinine of 1.1–2.1 mg/dL, (4) hypertension ( $N=2$ ). All five patients had normal ADAMTS13 levels and negative testing for Shiga toxin. Complement mutation genetic studies were obtained for four patients including 10 genes ( $N=2$ ) and 12 genes ( $N=2$ ) and were all negative. Testing for complement pathway including C5b-9 were obtained for 2 patients and were normal. All five patients were treated with eculizumab with induction treatment at 900 mg weekly  $\times 4$  doses, followed by one dose of 1200 mg on the fifth week, and 1200 mg every 2 weeks thereafter. Patients had a recovery of hemoglobin and platelets and a rise in haptoglobin and a normalization of LDH within 4–6 weeks from the start of eculizumab. Eculizumab was discontinued for 3 of the 5 patients without recurrence of their TMA; they are now 18–24 months since the discontinuation of eculizumab. In summary, there is a subacute syndrome of thrombotic

microangiopathy that can occur late post transplant. This syndrome appears to be complement mediated as shown by its response to a terminal complement inhibitor. It also appears to be transient without recurrence following treatment discontinuation.

**Disclosure of conflict of interest:** None.

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**Donor complement gene abnormalities cause transplant associated microangiopathy after allogeneic bone marrow transplantation**

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Transplant associated microangiopathy (TAM) is a very severe complication occurring after allogeneic bone marrow transplantation (BMT), burdened by a high case-fatality rate. It is characterized by abnormal complement activation, triggered by various agents (calcineurin inhibitors, acute GvHD, infections) with subsequent endothelial damage. In the literature, 6 cases of mutations in recipient complement genes are described, but none in donor DNA. Here we describe for the

first time 3 patients affected by TAM, carrying mutations in donor complement genes. In our lab, we studied 6 patients affected by TAM; they were screened for CFH autoantibodies, ADAMTS13 function and variants and macro-rearrangements in CFH (and related), CFI, CFB, CD46, C3, DGKe, THBD genes and at-risk haplotype (CFH-H3 and MCPggaac) by means of next-generation sequencing (NGS) and Multiplex Ligation-dependent Probe Amplification Analysis (SALSA MLPA P236 ARMD mix-1; MRC Holland). NGS was used to sequence DNA by Haloplex kit (Agilent) on a Miseq (Illumina) platform with >50-fold coverage of every target base. The bioinformatic analysis was performed using Sophia Genetics and the pathogenicity was assessed by means of *in silico* predictions (Polyphen2, SIFT, MutationTaster, AlignGVGD). All of the predicted pathogenetic variants were confirmed using Sanger sequencing. The same genetic screening was extended also to donor DNA in all 6 cases. The screening for known causes of TAM revealed mutations in recipient complement genes in one case; no mutations were found neither in recipient nor in donor DNA in two cases; instead, donor genetic alterations were found in 3 patients whose characteristics are summarized in Table 1. In particular, in patient 1 we found a well-known pathogenetic heterozygous mutation of CFH (c.1548T>A; p. Asn516Lys-MAF in European-Non-Finnish according to ExAC: 0.0004), a Variant of Uncertain Significance (VUS) in CFHR5 p. Arg356His and a homozygous at-risk haplotype CFH-H3. In patient 2 a heterozygous frameshift variant in CFHR5 c.485\_486dupAA (p.Glu163Lysfs\*10 - MAF 0.0065 in European-Non-Finnish ExAC) was found in donor DNA. In patient 3, genetic screening revealed that donor DNA carried a VUS in the gene encoding for complement component 3 (c.463A>C; Lys155Gln-MAF 0.0059 in European-Non-Finnish ExAC). Two patients were treated with Eculizumab and experienced a remission of TAM without residual organ damage, instead one patient obtained spontaneous TAM remission. Our results show that TAM can occur also in patients in which complement mutations are harbored by

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	Patient 1	Patient 2	Patient 3
Sex	F	F	F
Age	21	15	55
Disease	Burkitt Lymphoma	Blackfan-Diamond Anemia	Non Hodgkin Lymphoma
Donor	MUD	MUD	MUD
Time from BMT to TAM	6 months	6 months	1 month
Diagnosis of TAM	Renal biopsy	Clinical and laboratory findings	Clinical and laboratory findings
Donor complement gene abnormalities	1.Heterozygous point mutation in CFH  2.VUS in CFHR5  3.Homozygous at-risk haplotype for CFH-H3	Heterozygous point mutation in CFHR5	VUS in C3
Patient outcome	TAM spontaneous remission with severe residual renal impairment	Treatment with Eculizumab, remission of TAM	Treatment with Eculizumab, remission of TAM

Table 1:- Clinical and genetic characteristics of patients affected by TAM carrying abnormalities in donor complement genes

donor hematopoietic cells. In the three cases presented, TAM was relatively delayed with respect to HSCT, in particular in two cases (6 months) and this timing is compatible with the concept of reticulo-endothelial 're-population' by donor cells of monocytic lineage, responsible for the production of regulatory proteins of the alternative pathway of the complement. We also underline the response to anti-C5 inhibition in the 2 patients who were treated with Eculizumab; this fact further supports the hypothesis that the disease was related to complement dysregulation. We therefore suggest that both the recipient and the donor should be screened for complement gene mutations, so that more cases could be identified and the pathogenesis of TAM could be further clarified.

**Disclosure of conflict of interest:** G Ardissono is a SAB member of the HUS Global Registry sponsored by Alexion Pharma INC. The remaining authors declare no conflict of interest.

### P325

#### **Donor-specific anti-HLA antibodies and primary graft failure risk in mismatched hematopoietic stem cell transplantation**

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Detection of donor-specific anti-HLA antibodies (DSA) has been reported to be associated with graft failure (GF) in mismatched HSCT, but their frequency and their clinical impact remain unclear. We prospectively evaluated the presence of DSAs, using Solid Phase system, Luminex Commercial Kits (Labscreen Mixed and Single Antigen class I and II, One Lambda), in adult patients undergoing unmanipulated unrelated mismatched SCT (USCT) and unmanipulated haploidentical SCT (HaploSCT). DSAs binding level was expressed as mean fluorescence intensity (MFI). Twenty-six consecutive patients that underwent HSCT between May 2015 and October 2016, and with a post-HSCT follow-up of at least 1 month, were analyzed: 9/26 (35%) underwent one HLA minor or major mismatch USCT and 17/26 (65%) HaploSCT. Patients were affected by AML (11 pts), ALL (5 pts), HL (3 pts), MM (3 pts), MDS (2 pts), CML (1 pts) and MF (1 pts). USCT conditioning was Busulfan or Fludarabine-based and GvHD prophylaxis Cyclosporine, ATG and Methotrexate. HaploSCT conditioning regimen was based on Thiotepa, Busulfan and Fludarabine and GvHD prophylaxis on Cyclosporine, Mycophenolate and post-transplant Cyclophosphamide. DSA were detected in 5 patients (19%) with a median DSA level of 3700 (range: 765–22 400): 4 patients underwent HaploSCT and 1 USCT. Four of them did not receive desensitization treatment before transplant and failed to obtain allogeneic engraftment. Among these we observed one autologous engraftment, one death due to septic shock before engraftment and two primary GF. We used a desensitization treatment based on 4 plasma exchange procedures, intravenous immunoglobulin (1 g/kg) and Rituximab (375 mg/sm) in 2 patients. One of these patients (AML, haploidentical donor) had DSA against HLA-B50 (MFI 900). She experienced primary GF with increasing titles of DSA (maximum MFI 10 500); so, on day 38, a second transplant from the same donor was performed after a desensitization treatment. A progressive decrease in DSA was documented (up to MFI ≤ 200). On day 12 patient achieved neutrophil count over 500/μl and on day 23 platelet count over 20 000/μl. The second patient (MDS, haploidentical donor), instead, received a desensitization procedure before the first transplant. She had DSA against HLA-A24 (MFI 3700), and after

desensitization DSA levels decreased and reached 0. On day 20 patient achieved neutrophil count over 500/μl and on day 38 platelet count over 20 000/μl. DSA were detected in 1/9 of USCT candidates (11%) and 4/17 of HaploSCT candidates (24%) and they were associated with failure to obtain allogeneic engraftment in 3 cases. Desensitization treatment achieved DSA clearance and engraftment in the 2 patients in which it was performed, underlining the potential benefit of this procedure in the setting of HSCT with DSA that has to be validated by prospective and controlled studies.

**Disclosure of conflict of interest:** None.

### P326

#### **Early complications and late effects and quality of life at myeloma multiplex patients**

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The subject of this research is the quality of life at patients with Myeloma Multiplex at diagnosis and during therapy within 6–12 months. The research aims to analyze patients to be able to continue activities which will contribute for improving their quality of life as a priority task placed before the patient, his family, health institutions and social environment. This research was conducted at the University Clinic for Hematology Skopje in the period from June 2009 to March 2012. It covers patients infected with multiple myeloma, diagnosed and treated during this period. A total of 80 patients analyzed, using the EORTC QLQ C30 ver. 3.0 standardized questionnaires for HR quality of life that analyzed the physical, cognitive, emotional, personal and social functions related to the patients. It also analyzed and general health and quality of life. Analysis of physical functioning at diagnosis is 27.5 during treatment 59.5, significantly improved. Personal functioning at patients at the diagnosis is 17.9, during therapy – 36.4. Analyzing emotional functioning in patients at diagnosis is 39.9, during the therapy over 73.3 significantly improved. In examining the cognitive functioning is also a significant difference at diagnosis 55.2, during treatment 72.5. Social functioning of the patients was 26.2 at the diagnosis; during the treatment grow to 50.8. Significant improvement was notices in these patients' symptoms like fatigue, nausea and vomiting, pain, dyspnea, insomnia, loss of appetite, constipation and diarrhea. The analysis of the financial difficulties of patients at diagnosis is 76.2 and 72.5 during treatment, meaning no significant difference in the time given. The analysis of the overall health and quality of life at patient has a value of 23.9, and during therapy 58.8. Quality of life at patients with myeloma multiplex that makes the research group was significantly improved as a result of on time diagnosis and treatment with modern medicaments and the role of social worker with the application of certain social skills, continuous counseling, guidance and education for their reintegration in the community. Installing the quality of life as a separate category and investigating the factors that affect its expression in the daily functioning of the patients within the changed framework of action, as like this example for malignant disease. The needs of clearly defined interactions patient illness and treatment, quality of life and specifying the segments where it can effectively act and improve in order to achieve positive progression towards improving the qualitative features of this category is a clear and primary objective that must be inserted into the current approaches to monitoring patients with malignant hematological diseases.

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**Disclosure of conflict of interest:** None.

**P327****Early hyperglycaemia after initiation of glucocorticoid therapy predicts adverse outcome in patients with acute graft-versus-host disease**

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Acute graft-versus-host disease (aGvHD) is a common and severe complication after allogeneic stem cell transplantation. Since the current first-line treatment is based on treatment with systemic glucocorticoids (GC), steroid-induced hyperglycaemia develops frequently in patients with (aGvHD) potentially impacting on their outcome. We performed a retrospective analysis on 104 patients who received systemic GC for aGvHD and thoroughly investigated the consequences of aberrant glucose metabolism. In particular, we focused on glucose parameters early after initiation of GC. With a median of 50 (range: 4–513) blood glucose measurements during GC treatment, increasing mean, median and maximum glucose levels as well as the need for insulin treatment were associated with decreased overall survival (OS) in simple and multiple survival analysis. Early hyperglycaemia, as defined by mean blood glucose levels > 125 mg/dl during the first 3 days of GC therapy, was also found to be highly associated with adverse outcome: in multivariate analysis, the hazard ratio (HR) for death was 2.5 (95% CI 1.32–4.87,  $P=0.005$ ) in patients with early hyperglycaemia. While the risk of death due to relapse was not increased, the HR for death due to non-relapse mortality was 3.26 (95% CI 1.53–6.92,  $P=0.0021$ ) in a competing risk analysis. A score based on early hyperglycaemia and non-response to GC within 7 days allowed the identification of three risk groups: patients with both risk factors had an inferior OS at 5 years of 4.1% as compared to 75.4% in patients with none. Patients with one risk factor had a 5-year OS rate of 32.0% ( $P=0.0002$  for trend). In this retrospective study, we identified early hyperglycaemia after GC initiation as a prominent factor predicting increased non-relapse mortality in aGvHD patients. In addition, a score based on early hyperglycaemia and lack of response to GC was highly predictive for overall survival in these patients.

**Disclosure of conflict of interest:** None.

**P328****Early toxicity because of infectious complications not relapse is the main cause of death after allogeneic transplantation in aplasia for patients with refractory or relapsed acute myeloid leukemia**

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Patients (pts) with acute myeloid leukemia (AML) and induction failure or relapse have a dismal prognosis. An early allogeneic transplantation after reduction of blast count has shown promising results with an overall survival between 20 and 40%. In 2009 we started to offer an allogeneic transplantation (tx) in aplasia to all eligible patients with an HLA-compatible donor as soon as possible after diagnosis of refractory or relapsed AML. 31 pts (median age 51; 24–71) received an allogeneic tx after induction of aplasia. Diagnoses were primary AML in 26 pts, t-AML in 3 and s-AML in 2 patients. 25 pts were transplanted because of primary refractory AML, in 6 pts the indication was relapsed AML. ELN classification was favorable in 4 (all NPM pos), intermediate-1 in 8, intermediate-2 in 5 and adverse in 14 pts. First induction therapy consisted of daunorubicin and Cytarabine (3+7) in all but one pt. A second induction cycle with

high-dose Cytarabine was given in 13/25 pts with induction failure. The search for a stem cell donor was started immediately after results of high-risk cytogenetic, no achievement of bone marrow aplasia on day 14 of induction therapy, or immediately after diagnosis of relapse. Four patients had a related 10/10 donor, for 17 patients a 10/10 matched unrelated donor was identified and 10 patients received a transplant from a 9/10 unrelated donor. The interval between diagnosis of primary disease or relapse and tx was 3 (1–7) months (mo) for both groups. In 24 patients melphalan (100–140 mg/m<sup>2</sup>) was used to induce an aplasia before starting conditioning therapy. The interval between melphalan and conditioning therapy was 13 (9–21) days. Three pts started the conditioning therapy while in aplasia after previous chemotherapy. The conditioning therapy was of reduced intensity in all pts. and consisted of Treosulfan (30 g/m<sup>2</sup>)/Fludarabine (Flu) in 19 pts, TBI(8Gy)/Flu in 7 pts and Busulfan(8 m/(kg))/Flu in 5pts, respectively. ATG was given to all pts with an unrelated donor. Most pts (21/31) had a severe neutropenia with a median of 0.3/nl (0.1–5.2) before starting melphalan because of refractory leukemia. After a median follow-up of 21 (4–68) mo 11 pts (35%) were alive without relapse. 6 (19%) pts died because of a relapse after a median of 6 (3–25) mo. The non-relapse mortality was 45% (14/31 pts). Most of these pts (10/14, 71%) died because of infectious complications early after transplantation (med 1; 0–19mo). In 4 pts graft versus host disease was the main cause of mortality. In this retrospective 'real-life' analysis, we showed that an early allogeneic transplantation is feasible for patients with primary refractory or relapsed AML. A reduced intensity conditioning after induction of aplasia with melphalan offers a chance of long-term relapse-free survival for about 30% of patients with an otherwise dismal prognosis. NRM is high, especially because of infectious complications early after transplantation, probably related to the long period of severe neutropenia. Therefore, the focus has to be set on early recognition and intervention of infectious complications.

**Disclosure of conflict of interest:** None.

**P329****Eculizumab treatment of transplantation-associated thrombotic microangiopathy: added value of timely determined terminal complement activity and eculizumab drug level monitoring**

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Recent evidence supports the effector role of complement activation in several types of thrombotic microangiopathy — the atypical hemolytic uremic syndrome (aHUS) as well as the transplantation-associated thrombotic microangiopathy (TA-TMA). The blockade of the terminal complement complex formation by anti-C5 monoclonal antibody eculizumab provides an effective treatment option in severe and devastating cases of TA-TMA. The experience with the use of eculizumab in this indication is slowly accumulating in the HSCT community, however the published data originate from small case series or uncontrolled trials and sharing of emerging real-life observations may be valued. On case reports of two pediatric patients treated with eculizumab for TA-TMA with very detailed follow-up of multiple complement parameters, including terminal complex sC5b-9 and eculizumab drug levels we would like to demonstrate: (1) Achieving therapeutic levels of eculizumab (> 99 µg/ml) may be unsuccessful even with intensified dosing interval. Furthermore, we documented rapid eculizumab clearance from circulation which allowed only for short periods (< 48 h) of efficient drug levels during the

weekly dosing. (2) We did not observe tightly correlated sC5b-9 and eculizumab levels within the dosing intervals; however the long-term sC5b-9 formation suppression was achieved concomitantly with improved eculizumab levels and slowed drug clearance. (3) Classical complement pathway activity assay (CH50) may not reliably substitute for therapeutic efficiency monitoring in case of hypocomplementaemia due to protein losses (profound diarrhea, proteinuria, GI bleeding, catabolism). This holds true also for the alternative pathway activity which remained low during treatment in both patients. (4) Mycotic infections may represent serious therapy related risks in eculizumab treatment after HSCT (both patients achieved control of complement activation after multiple doses of eculizumab, however suffered fatal infections subsequently). Besides, we observed a significant increase in C3a concentrations correlated with clinical onset of infection which invites for further investigation of this complement cascade product as early indicator of mycotic infection. In conclusion, we would like to highlight the great added value of timely available complement assay results, including sC5b-9 and especially eculizumab drug level values—to be used together with detailed clinical parameters for directing effectively these highly personalized (and also costly) treatments.

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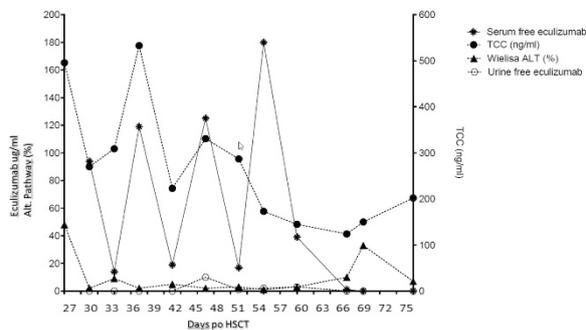


Fig. 1: Dynamics of sC5b-9 (TCC), eculizumab levels and alternative pathway activity in Patient 1.

**Disclosure of conflict of interest:** None.

[P330]

Results	ZARZIO® N=180 (Mar-13 / Nov-16)	MYELOSTIM® N=99 (Jan-09 / Feb-13)	NEULASTA® N=60 (Mar-06 / Dec-08)	P
Hematologic recovery, days (range) ANC > 0.5 × 10 <sup>9</sup> /L PLT > 20 × 10 <sup>9</sup> /L	11 (8-30) 15 (9-120)	11 (9-29) 14 (10-35)	10 (8-18) 12 (9-23)	<b>0.001</b> <b>0.007</b>
Median G-CSF injections, days (range)	9 (4-26)	9 (4-26)	-	0.854
FUO in neutropenia episodes (%)	27 (15%)	10 (10.1%)	13 (21.7%)	0.119
Microbiologically documented infections (%)	67 (37%)	43 (43.4%)	19 (31.7%)	0.524
Intravenous antibiotics needing (%)	94 (52%)	53 (53.5%)	32 (53.3%)	0.692
RBC transfusions (Mean ± SD)	0.74 ± 1.82	0.78 ± 1.47	0.44 ± 0.95	0.121
Median PLT transfusions (range)	2 (0-13)	2 (0-12)	1 (0-6)	<b>0.001</b>
Median hospitalization duration, days (range)	21 (14-49)	24 (15-68)	21 (6-29)	0.202
TRM (%)	2.2%	2%	1.7%	0.809

### P330

#### Efficacy and safety of biosimilar Filgrastim (Zarzio) after autologous stem cell transplant: a prospective study with historical comparison with Lenograstim and Peg-Filgrastim

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Biosimilar Filgrastim Zarzio has been approved for autologous peripheral stem cell mobilization and for prophylaxis of severe neutropenia duration following conditioning chemotherapy and stem cell infusion. However, there is still skepticism about safety and efficacy of Zarzio in this setting, considering the lack of prospective studies with a long follow-up. From March 2013 to November 2016, 180 consecutive adult patients with hematologic malignancies (plasma cell disorders  $n=101$ ; Non Hodgkin and Hodgkin's lymphomas  $n=75$ ; others  $n=4$ ) underwent autologous stem cell transplant (ASCT) in our Institution. Zarzio was given at the dosage of 5 mcg/kg beginning to day 3 from infusion of stem cells and continued until neutrophils recovery, with the aim to evaluate the efficacy and the safety of this biosimilar G-CSF. Hematologic recovery was defined as an absolute neutrophils count upper than  $0.5 \times 10^9/L$  and a platelets count upper than  $20 \times 10^9/L$  in three consecutive checks. This cohort of patients was compared with two historical cohorts in our Institution: (a) 99 consecutive adult patients treated with Lenograstim (Myelostim) at dosage of 5 mcg/kg daily given from day 3 after infusion from January 2009 to February 2013; (b) 60 consecutive adult patients treated with peg-filgrastim (Neulasta) at dosage of 6 mg single dose at day 3 after infusion from March 2006 to December 2008. The three patient cohorts were similar for all baseline features analyzed, without any significant differences in terms of sex, median age, diagnosis, median chemotherapy lines prior ASCT, disease status at ASCT, conditioning regimen and median infused CD34+ cells. Clinical results of patients are resumed in Table 1.

No difference in terms of drug-related adverse events was observed in the three patient cohorts with no reported serious adverse events. Similar results were obtained performing two separate sub-analysis only for lymphoma or myeloma patients. Despite the limitations due to the non-randomized nature of the study, from our data on a large cohort of patients

with a long-term follow-up biosimilar Filgrastim (Zarzio®) could be considered substantially equivalent in terms of efficacy and safety to Lenograstim (Myelostim®) and Peg-Filgrastim (Neulasta®), when used for hematological recovery and febrile neutropenia prophylaxis after ASCT in adult patients with hematologic malignancies.  
**Disclosure of conflict of interest:** None.

**P331**  
**Eltrombopag induces responses in post allo-HSCT poor graft function**

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Poor graft function (PGF) is a no desirable post allo-HSCT condition. It is diagnosed in pts with two or three cytopenias (Hgb < 10 g/dl, ANC < 1.0×10<sup>9</sup>/L, platelets < 30×10<sup>9</sup>/L) at day +30 post-transplant, with transfusion requirements, associated with hypo/aplastic BM, in the presence of complete donor chimerism and in the absence of severe GVHD and relapse (Yang *et al.*). PGF is more frequent in alternative allo-HSCT types, such a haplo-identical, mismatched, or umbilical CB. Several therapeutic approaches, with poor results, have been tried. Recently, eltrombopag (EPAG) has been shown to improve platelet count in the post allo-HSCT setting (Popat *et al.*, ASH 2015). In this retrospective observational study, we analysed the efficacy of EPAG in pts with post-transplant PGF. We studied all 107 adults who underwent allo-HSCT during a 23-month period (01 January 2015 to 22 November 2016) in our Center. A total of 6 pts (5.6%) received EPAG for PFG with thrombocytopenia. Three pts were male, and three female. Median age was 59 years (24–67). The baseline diagnoses were: ALM (2), MDS-RAEB (1), idiopathic myelofibrosis (1), AA (1), and CLL (1). Three transplants were from family donor (all of them haplo-identical), and 3 from unrelated donor (the three of them HLA 9/10). SC source was PB in 5 cases, and BM in 1. EPAG was started at 50 mg/day and escalated each 2 weeks to 75, 125 and 150 mg if platelet count was < 50×10<sup>9</sup>/L. We analysed the platelets, ANC, and Hgb at EPAG initiation and 90 days after being with the maximum dose. Median time between allo-HSCT and eltrombopag

initiation was 120 days (17–155). Median maximum dose used of EPAG was 150 mg/day (125–150). Median platelets, ANC and Hgb before starting treatment were 13×10<sup>9</sup>/L (5–28), 1×10<sup>9</sup>/L (0.07–11.2) and 8.6 g/dl (7.6–12.1), respectively. Five patients (83%) were severely thrombocytopenic (platelet count ≤ 20×10<sup>9</sup>/L), 4 (67%) were anemic (Hgb < 10 g/dl), and 3 (50%) were neutropenic (ANC < 1.0×10<sup>9</sup>/L). Median platelets, ANC and Hgb at day +90 of maximum dose were: 37×10<sup>9</sup>/L (8–108), 2.4×10<sup>9</sup>/L (0.93–9.62) and 11.8 g/dl (7.9–14.5), respectively. The 5 thrombocytopenic pts (100%) responded to EPAG, with increases of 120 00, 25 000, 28 000, 39 000 and 96 000×10<sup>9</sup>/L in the platelet count. Three anemic pts (75%) responded and achieved increases of Hgb of 1.1, 4.7 and 6.8 g/dl. Finally, the 2 neutropenic pts (66.6%) responded and achieved increases of ANC of 4080 and 9550×10<sup>9</sup>/L. At the moment of the analysis close, pts are at a median of +11.5 months post-HSCT (8–16), and all but one (who died from a septic shock) are alive and outpatient. This survival is striking for subjects who develop a complication with such a high expected mortality as PFG.

PGF is a life-threatening complication, relatively frequent after alternative donor HSCT, whose treatment has been very disappointed. We report our experience in pts who developed PGF during the last 2 years. EPAG induced responses in platelets in all pts of the studied group. Bilineal and trilineal responses were also seen. In our opinion, prospective studies are warranted in order to confirm EPAG as a new efficient treatment of post-HSCT poor graft function.

**Disclosure of conflict of interest:** None.

**P332**  
**Engraftment syndrome (ES): identification of risk factors**

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Engraftment syndrome (ES) is considered an important complication after autologous stem cell transplantation (ASCT). However, most cases of ES are mild and resolve spontaneously or with corticosteroid therapy. Objectives: Detection of risk factors of ES development in patients who underwent ASCT in the last 3 years in our center. A retrospective case-matched analysis was performed compar-

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Patient	Age (years)	Disease	SC	Type of HSCT	Conditioning	Plat pre-EPAG (10 <sup>9</sup> /l)	Plat post-EPAG (10 <sup>9</sup> /l)	Hgb pre-EPAG (g/dl)	Hgb post-EPAG (g/dl)	ANC pre-EPAG (10 <sup>9</sup> /l)	ANC post-EPAG (10 <sup>9</sup> /l)
1	59	CLL	PB	Haplo-id (Cy post)	Flu-Cy-Bu 2	10	49	10	10.2	0.32	4.4
2	58	Myelofibrosis	PB	Haplo-id (Cy post)	Flu-Cy-Bu 2.5	15	40	8.6	13.3	11.2	1.7
3	60	AML	PB	Haplo-id (Cy post)	Flu-Cy-Bu 2	5	33	7.7	14.5	0.07	9.62
4	66	AML	PB	URD (9/10)	Flu-Bu 2	28	8	7.6	7.9	0.9	0.93
5	24	AA	BM	URD (9/10)	Flu-Cy-ICT 2	17	29	8.6	9.7	1.1	1.7
6	67	MDS-RAEB	PB	URD (9/10)	Flu-Bu 2	12	108	12.1	13.7	2.59	3.13
					Median (range)	13.5 (5-28)	36.5 (8-108)	9.3 (8.6-10)	11.8 (7.9-14.5)	1.0 (0.07-11.2)	2.42 (0.93-9.62)

[P332]

Characteristic	Engraftment syndrome	Control Patients	p Value
	Median	Median	
Engraftment day	12 (12-23)	12 (10-15)	0,085
Number of CD34+ cells/kg infused	2,65 (1,90-7,22)	3,4 (2,03-10,40)	0,043
Day of onset of symptoms	12 (8-17)	7 (3-13)	0,0001
(CSF-G) therapy	7 (4-18)	6 (5-11)	0,075
Weight gain	7,5 (3-17)	6 (2-14)	0,007
Maximum serum GOT	49,5 (26-272)	29 (28-70)	0,0001
Basal serum GOT	16,5 (9-27)	26 (14-28)	0,176
Maximum serum GPT	66,5 (10-438)	43 (28-70)	0,001
Basal serum GPT	22 (8-32)	28 (8-36)	0,751
CRP	9,4 (0,20-25,40)	13,1 (2,20-27)	0,851
PCT	0,62 (0,10-9,10)	0,45 (0,12-0,77)	0,251

ing 19 patients who developed ES with an equal number of patients who did not between January 2013 and November 2016. We analyzed variables such as CD34+ cells per kg infused, use of granulocyte colony-stimulating factor (CSF-G) and engraftment day. Analytical data, including baseline and maximum determination of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), C-reactive protein (CRP) and procalcitonin (PCT), as well as clinical data fever, weight gain, digestive and respiratory symptoms, pulmonary infiltrates were analyzed. Sixty-eight patients were women. Median age was 59 years old (range: 39–73). Patients were conditioned with BEAM (52%), Melphalan 200 mg/m<sup>2</sup> (42%) and BCNU-TT (5%). Nineteen patients developed ES in our series, which correspond to eight percent of all ASCT. Case and control groups were matched according to age, sex, diagnosis and conditioning regimen. The most prevalent baseline disease in the group with ES was Myeloma (42.1%), followed by Mantle cell lymphoma (26.3%). All patients who developed ES had fever, 79% skin rash, 37% respiratory symptoms, 16% pulmonary infiltrate and 9% digestive symptoms. A summary of the comparison of data analyzed in subgroups is shown in Table 1. We found significant difference in the percentage of weight gain ( $P=0.007$ ), increase of TGO ( $P=0.0001$ ), increase of TGP ( $P=0.001$ ) and increase the number of CD34+ cells per kg infused ( $P=0.043$ ), we found an inverse correlation between the number of CD34+ cells per kg infused and incidence of ES. However, in terms of post-transplant CSF therapy ( $P=0.075$ ) and CRP and PCT valor ( $P=0.85$  and  $P=0.25$ , respectively) we did not find significantly difference to develop ES. In our series, weight gain and TGO and TGP rise were risk factors for ES development. Therefore, we should be aware of ES in patients who develop fever, elevated liver enzymes and weight gain during graft phase. We did not find a significant difference in CRP and PCT suggested in other studies. Further studies are required to better characterize risk factors of ES development.

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#### P333

##### Evolution of weight and nutritional parameters in patients undergoing hematopoietic stem cell transplant

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HSCT is the only potentially curative approach for a number of diseases. Nutrition is an important issue during the process and the knowledge of the expected weight lost and evolution of the nutritional serological parameters for the majority of patients is important for the outcome of the procedure. This study includes the last 400 consecutive pts who underwent HSCT in our center (223 allo-HSCT, 177 auto-HSCT) (January 2012–October 2016). 214 pts were male (53.5%) and 186 female (46.5%). Median age was 54 years (range: 2–72). Baseline diseases were: acute leukemias (132), plasma cell dyscrasias (105), lymphoproliferative disorders (95), myelodysplastic syndromes (23), chronic myeloproliferative diseases (20), bone marrow failures (8), and others (17). Among the allo-HSCT cases, donor was unrelated in 123, and related in 100 (including 25 haplo-identical). Conditioning regimen was:

	Allo-HSCT (N=233)	Auto-HSCT (N=177)
Height (cm)	168 (125-198)	167 (86-189)
Weight at admission (kg)	72 (34-110)	75 (10-134)
Weight at hospital discharge	<b>68 (30-102)*</b>	<b>71 (10-127)*</b>
Weight at day +100	<b>66 (35-100)*</b>	<b>70 (13-133)*</b>
Albumin at admission (g/L)	41 (20-51.1)	43.2 (18.8-50.7)
Albumin at discharge (g/L)	<b>37.3 (13-50)*</b>	<b>35.7 (22.6-48.4)*</b>
Albumin at day +100 (g/L)	41.7 (21.7-49.5)	43.7 (12.8-50)
Pre-albumin at admission (g/L)	0.20 (0.01-0.47)	0.23 (0.05-0.35)
Pre-albumin at discharge (g/L)	<b>0.19 (0.08-0.40)*</b>	<b>0.14 (0.03-0.39)*</b>
Pre-albumin at day +100 (g/L)	0.23 (0.03-0.49)	<b>0.21 (0.05-0.34)</b>

Values in median (range); \*  $p < 0.05$ . In spite of the nutritional support, considerable loss of weight is a fact in the majority of patients undergoing allo and auto-HSCT. Basic nutritional parameters (as albumin and pre-albumin) are also affected and help the medical team to take care of nutritional status of HSCT patients. KEYWORDS: Weight, albumin, pre-albumin.

busulphan-based (155), melphalan-based (122), BEAM (58), TBI-based (22), and others (43). Weight at hospital discharge was significantly lower than at admission (5.6% in allo-HSCT, and 5.4% in auto-HSCT). Weight at day +100 was also significantly decreased compared with the admission (8.6% in allo-HSCT, and 6.7% in auto-HSCT). Weight at day +100 was lower than the ideal for their sex and height in the allo-HSCT setting. Contrarily, among the patients undergoing auto-HSCT, the weight at day +100 remained higher than the ideal for their sex and height in a high proportion of cases. Regarding serum albumin, it was significantly decreased at discharge (9% in allo-HSCT, and 17.5% in auto-HSCT), but recovered values similar to admission at day +100. In the auto-HSCT setting, prealbumin levels were significantly reduced at discharge (39%), and in lower proportion at day +100 (8%), compared with admission values. In the allo-HSCT patients, prealbumin levels were significantly reduced at discharge (5%), but had been recovered at day +100, compared with admission values. **Disclosure of conflict of interest:** None.

### P334

#### Factors predicting graft versus host disease-free, relapse-free survival after allogeneic stem cell transplantation: comparison attending to two different definitions of 'chronic graft versus host disease event'

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Disease free survival is the most common used endpoint for clinical research on allogeneic stem cell transplantation (HSCT), but it doesn't include morbidity endpoints or those which affect their quality of life as graft versus host disease (GVHD). Recently, Blood and Marrow Transplant Clinical Trials Network has proposed a composite endpoint: GVHD-free, relapse-free survival (GRFS) for HSCT outcomes. This endpoint includes as event: III-IV acute GVHD (aGVHD), relapse, death or chronic GVHD (cGVHD) requiring systemic treatment. In the last EBMT annual meeting a redefinition of this endpoint was proposed

changing cGVHD event from those patients with cGVHD requiring systemic treatment (the original one) to those with just severe cGVHD (the redefined one). We retrospectively analysed 603 patients consecutively transplanted (1995–2014) excluding non-malignant diseases, second allo-SCT and those < 16 years old age. We had generated two composite endpoints: in both III–IV aGVHD, relapse or death were considered events but we defined GRFS1 as the one with cGVHD event including those who required systemic treatment (as the original one) and in GRFS2 just those with severe cGVHD (the EBMT redefined one). The median age was 49 years (16–69) and 59% (362) were males. Other characteristics of patients are resumed in Table 1. With a median follow up for patients alive of 39 months (3–179), the median estimated survival in months and the % at +1 year and +2 years was: 114 months, 71% and 62% overall survival (OS); 24 months, 58% and 50% event free survival (EFS); 6 months, 35% and 26% GRFS1; 11 months, 46% and 38% GRFS2. 138 (23%) and 210 (35%) hadn't any event in GRFS1 and in GRFS2, respectively. In GRFS1, event's incidence was: 90 (15%) for III–IV aGVHD, 170 (28%) for cGVHD, 151 (25%) for relapse and 54 (9%) for death; In GRFS2 was 90 (15%), 65 (11%), 173 (28%) and 65 (11%), respectively. Considering those patients with cGVHD as event in GRFS1, 105 of them hadn't the event as cGVHD at the same time in GRFS2 (since they had cGVHD requiring systemic treatment but not severe cGVHD). For these patients, the alternative event in GRFS2 was: 72 without any event, 22 relapsed and 11 died. In the multivariate, the factors associated with better outcomes were: in GRFS1 early EBMT stage ( $P < 0.001$  with early as reference; intermediate  $P = 0.016$ , HR 1.36, 95% CI 1.06–1.75; advance  $P < 0.001$ , 1.74, 1.3–2.3), *in vivo* T-cell depletion ( $P = 0.02$ , 0.57, 0.26–0.96) and haploidentical ( $P = 0.04$  with HLA identical as reference, no significance 1 or 2 mismatch ( $P = 0.18$ ), haploidentical  $P = 0.038$ , 0.5, 0.26–0.96) but only early EBMT disease stage maintained it in GRFS2 ( $P < 0.001$  with early as reference; intermediate  $P = 0.011$ , 1.4, 1.09–1.88; advance  $P < 0.001$ , 1.87, 1.4–2.5). In our study the percentage of the GRFS end point was similar to previously reported. Comparing both proposed definitions, the GRFS2 end point define a population of patients without any event; it is possible that the morbidity is misdiagnosed in this group. The EBMT disease score was the

factor with more impact in both, but it is interesting to point it out that haploidentical donor had an advantage in GRFS1. These results are being validated in a large series and the definitive results will be available at the moment of the meeting congress.

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<b>Table 1. Global series (n=603)</b>	<b>n</b>	<b>%</b>
Diagnosis		
• AML/MDS/MF	288	48%
• ALL	73	12%
• NHL/HL/CLL	149	25%
• CML	40	6%
• MM	53	9%
EBMT stage disease		
• Early	268	44%
• Intermediate	234	39%
• Advance	100	17%
Reduced intensity conditioning regimen	381	64%
Peripheral blood stem cell source	517	86%
GVHD prophylaxis		
• CSA/TCR + MTX	390	65%
• CSA/TCR + MMF	62	10%
• TCR/SIR	136	23%
• Others	13	2%
<i>In vivo</i> T cell depletion	54	9%
Donor:		
• Sibling HLA identical	379	63%
• Sibling 1 or 2 mismatch	14	2%
• Haploidentical	23	4%
• Unrelated HLA identical	106	18%
• Unrelated 1 ó 2 mismatch	59	10%
• Cord blood donor	21	3%
aGVHD	369	61%
• Grades III-IV	94	16%
cGVHD	368	61%
• Moderate	140	23%
• Severe	84	14%

**Disclosure of conflict of interest:** None.

**P335**

**Previously published**

**P336**

**Hematopoietic stem cell transplant associated thrombotic microangiopathy and acute graft-versus-host disease**

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Steroid refractory acute graft-versus-host disease (GvHD) remains a major complication of allogeneic hematopoietic

stem cell transplantation (allo-HSCT). Affected patients have a very poor prognosis. GvHD has been associated with transplant-associated thrombotic microangiopathy (TA-TAM). Endothelial damage mediated by radiation, viral reactivation, drug exposure or alloreactivity results in exposure of subendothelial collagen, activation of coagulation and small vessel occlusion to a degree that results in organ failure. Complement is thought to be a major mediator of endothelial damage. Although a consensus exists about the exceedingly high morbidity and mortality of TA-TAM and diagnostic criteria have been converging to a consensus, no biomarkers to diagnose TAM and predict outcome have been established. We hypothesize that a TA-TMA, related to dysregulation of the alternative complement pathway correlates with organ damage. A retrospective analysis of 660 consecutive patients with hematological malignancies receiving an allo-HSCT at the University Hospital Basel in the period from 2003 to 2013 was performed. Data on the occurrence, risk factors and outcome of patients with TA-TMA and the correlation with acute GvHD was collected. Available biopsies of organs suspected to be affected by TAM and/or GvHD will be performed. Routine bone marrow biopsies for histological, immunohistochemical signs of TA-TAM and complement activation will be analyzed. Serum samples will be used to characterize markers of complement activation using plasma levels of C5b-9 and C5b-9 deposition in tissues biopsies. 660 patients (AML *n* = 260; ALL *n* = 152; MDS/MPN *n* = 93; lymphoid neoplasm *n* = 85; plasma cell disorder *n* = 53; bone marrow failure *n* = 17) underwent myeloablative (*n* = 432) and non-myeloablative (*n* = 228) allo-HSCT at a median age of 47 years (range: 19–71 years). Forty-eight (7.3%) patients matched the established diagnostic criteria for TAM (increased LDH, platelet count < 50 g/L or < 50% of normal baseline, schistocytes > 2 per high power field, creatinine increase). The median time to onset of TAM was 36 days post-transplant (range: 22–67 days). Subjects with TA-TAM had significantly higher 3-year non-relapse mortality compared to those without (47.8% vs 18.2%, *P* < 0.001). Grades 2–4 aGvHD and cytomegalovirus viremia were independent risk factors for TA-TAM, and serum LDH level > 500 U/L as well as arterial hypertension were early signs of TA-TMA occurrence. Patients with clinically relevant aGvHD (≥ grade 2) had more TA-TAM than patients without aGvHD (45% vs 24%; *P* < 0.001). TAM correlated with aGvHD severity; the higher the aGvHD grade, the more the patients who suffered from TAM. Allo-HSCT recipients with grades 2–4 aGvHD or cytomegalovirus viremia should be closely monitored for the presence of TA-TMA. At the meeting first results of histological, immunohistochemical and complement activation analyses will be presented.

**Disclosure of conflict of interest:** None.

**P337**

**Hemorrhagic cystitis after unmanipulated haploidentical bone marrow transplantation with post-transplant cyclophosphamide**

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Hemorrhagic cystitis (HC) after stem cell transplantation (SCT) can cause significant morbidity and prolonged hospitalization. Early bleeding occurs almost exclusively when using Cyclophosphamide (Cy) (5–25% of cases), while late onset HC are classically attributed to BKV infection, and occurs up to 58% of patients (pts) receiving myeloablative haplo-SCT who had positive BK viruria (1, 2). We retrospectively studied HC cases among pts submitted to haplo-HSCT in our department. Thirty-eight pts receiving an haplo-SCT with post-transplant Cy (PT-Cy) were included (Table 1). Prophylaxis for Cy included hyperhydration (3 L/m<sup>2</sup> of 0.9% saline) and Mesna administration (200 mg for each 1000 mg of Cy/daily divided into three doses). Hematuria was graded as follows: grade I,

microscopic; grade II, macroscopic; grade III, with clots; and grade IV, leading to urinary retention or requiring surgical intervention (1). Pts with HC and clots were treated with continuous bladder irrigation. Twenty-three pts (60.5%) developed HC at a median of 9.5 days post-SCT (range: 1–57). Clinical severity was grade I in 6 cases (26.1%), grade II in 13 cases (56.5%), grade III in 2 cases (8.7%) and grade IV in 2 cases (8.7%). At the onset of HC diagnosis, BK viruria was investigated in 13/23 pts. Five pts (38.5%) had BKV negative (BKV–) HC and 8 pts (61.5%) BKV positive (BKV+) HC. BKV-HC occurred after a median of 5 days (range: 5–52) while BKV+ HC after 14.5 days (range: 5–57), respectively ( $P=0.06$ ). Among BKV+ pts, 4 received iv cidofovir 5 mg/kg once a week for 2 weeks and then once every 2 weeks. Median number of administrations was 3 (range: 2–4). Oral probenecid was given at the dose of 2 g 3 h before and 1 g 2 and 8 h after cidofovir administration. Two pts obtained a complete response (CR) after 70 and 110 days, respectively, one patient reached a partial response after 31 days and one pt failed to obtain a response. No pts developed renal toxicity during treatment. One pt received ganciclovir for concurrent CMV viremia and BKV+ HC resolved in 55 days. Three patients did not receive any treatment for mild or asymptomatic cystitis. All of them achieved remission after a median of 10 days from the onset (range: 5–57.) Among BKV-HC, 3 pts obtained spontaneous resolution after a median of 4 days (range: 1–52), while two pts died early after SCT. Finally, among pts for whom BK viruria was not available, a remission was reached in 6 of them after a median of 28.5 days (range: 12–43), while 4 pts died early after SCT. In our cohort of pts, HC occurrence was of 60.5% and BKV was responsible for the 61.5% of cases. Contrary to its high incidence, HC showed a relative benign course, with an overall remission rate of 87.5%, regardless of treatment. Finally, we found a trend for a longer interval between SCT and HC onset in pts with BKV+ HC, as compared to Cy-related HC ( $P=0.06$ ).

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[P337]

Table 1: Patients' features and transplant conditions

Patients 38	
Median age ys	52 (range, 21 to 71)
Sex	21 M/ 17 F
Underlying diseases	1 HL, 6 ALL, 23 AML, 1 NHL, 1 histiocytic sarcoma, 3 MFI, 3 MDS
Conditioning regimen	1 FLU-Cy, 1 FLU-TBI, 1 FLU-TT-MEL, 34 TT-BU-FLU
Stem cell source	Unmanipulated haploidentical bone marrow
Donor sex	25 M/13 F
GvHD prophylaxis	CSA+MMF and Cy 50 mg/Kg on day +3 and +5
Platelets engraftment	day 28 (range, 23 to 102); never reached for 10 pts

**Disclosure of conflict of interest:** None.

#### P338

##### High-dose methylprednisolone (HDMP) for the treatment of sinusoidal obstruction syndrome (SOS) in adults

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SOS is a rare and serious complication of hematopoietic stem cell transplantation (HSCT). It is diagnosed using the modified Baltimore criteria of hyperbilirubinemia, weight gain or ascites >5% over baseline, hepatomegaly or right upper quadrant pain of liver origin. Only Defibrotide has been approved for the treatment of veno-occlusive disease. HDMP has been described as effective SOS therapy in a few case series (1, 2). We describe our experience of treating adult SOS using HDMP. Objective is to retrospectively analyze the treatment efficacy and overall survival of patients diagnosed with SOS after HSCT and treated with HDMP. We used Vilnius University Hospital data base to identify patients diagnosed with SOS under Baltimore criteria and treated with HDMP over 2007–2016 period. Patient demographics, transplant and clinical data, response, survival (Kaplan–Meier survival analysis) and HDMP infusion related complications were analyzed. We identified 11 patients (9 males) of whom 10 had had allogeneic HSCT (6 reduced intensity conditioning) and one had received a double autologous HSCT. SOS was diagnosed on the median day +22 (+7 to +81 days). The median bilirubin value was 61.7 μmol/L (11.1–137 μmol/L). All patients had liver enlargement of median 210 mm (160–235 mm) on ultrasound. Two patients had normal bilirubin values but displayed the remaining signs and symptoms of SOS at diagnosis. Patients received intravenous methylprednisolone 500 mg/m<sup>2</sup> every 12 h for 3 days. None received defibrotide. Seven (64%) patients responded on median day +12 (+3 to +20 days) after the start of HDMP. Four responded by decrease in serum bilirubin by 50% and resolution of symptoms without the need of further treatment. The remaining three responders received maintenance treatment after one course of HDMP with reduced doses of methylprednisolone until resolution of symptoms. Four patients failed to respond and died of multiorgan failure on median day +12 (+5 to +41). The median observation time was 6 months (0–44 months). The median overall survival for the SOS group was 8 months (range: 0–18) and it was 27 months among the responders. No adverse reactions related to HDMP infusion were observed. HDMP therapy in adult SOS results in clinically relevant response rate. Further prospective trials are required to assess HDMP efficacy in comparison to defibrotide or as add on therapy.

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#### P339

##### Hypertension in children treated with hematopoietic stem cell transplantation

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Prevalence of hypertension (HT) in general pediatric population is ~4%, while in children treated with hematopoietic stem cell transplantation (HSCT) it is up to 30%. We assessed factors contributing to the development of HT in children treated with HSCT and usefulness of ambulatory blood pressure monitoring (ABPM) in this population of patients. The study included 30 children (21 boys, 9 girls; mean age 10.9 years) treated with HSCT for neoplasms ( $n=22$ ; 73%) or non-neoplastic disorders ( $n=8$ ; 27%). Control group included 19 children (8 boys, 11 girls; mean age 12 years). ABPM measurements (Spacelab device) were performed before HSCT and after a mean of 7 months after HSCT (in 16 of the 30 children). Blood samples were collected from 10 children treated with HSCT and all controls. Total RNA extraction was performed and microarray analysis was conducted using GeneChip Human Gene 1.0 ST Arrays (Affymetrix). In patients after HSCT no antihypertensive treatment was used. Mean systolic blood pressures (SBP) before and after HSCT did not differ significantly from the control group. Mean diastolic blood pressures (DBP) before and after HSCT were  $68.7 \pm 12.2$  mm Hg and  $65.4 \pm 9.42$  mm Hg, respectively, and mean DBP percentiles were  $77.1 \pm 7.9$  and  $78.3 \pm 6.7$ , respectively; the differences between the study group and the control group were significantly higher before HSCT. Mean 24-hour arterial pressure (MAP) percentiles were  $83.3 \pm 11.1$  and  $79.2 \pm 8.7$ , respectively; the differences between the study group and the control group were significantly higher before HSCT. Before HSCT and after the procedure, the European Society of Hypertension criteria for high normal blood pressure (BP) and HT were fulfilled in 16%/12% patients and 20%/0% patients, respectively. Nocturnal BP decrease  $< 10\%$  was found in 46%/53% patients and  $> 20\%$  nocturnal BP decrease in 3%/7% patients, respectively. In the control group  $< 10\%$  nocturnal BP decrease was found in 10% of children and  $> 20\%$  nocturnal BP decrease in 5% of children. When the groups of patients before and after HSCT were compared, highly significant differences were found in gene expression levels for MTHFR(5')-NPPB, MOV10, GRB14, SLC4A7, ULK4 0.07, MAP4, FGF5, SLC39A8, ENPEP, NPR3, C5orf23, BAT5, NOS3, GATA4, CACNB2(5') CACNB2(3'), C10orf107, PLCE1, FLJ32810, TMEM133, TBX5-TBX3, ULK3, FURIN-FES, GOSR2. Significant differences were also found between the group of patients after HSCT and the control group with respect to the following genes: MTHFR(5')-NPPB, ST7L, CAPZA1 7918569, MOV10, AGT 0.06, FIGN, SLC4A7, ULK4, MAP4, SLC39A8, ENPEP, NPR3, C5orf23, HFE 0.06, BAT2, BAT5, PIK3CG, NOS3, GATA4, CACNB2(5') CACNB2(3'), C10orf107, PLCE1, CYP17A1, NT5C2, ADM, PLEKHA7, FLJ32810, TMEM133, ATP2B1, SH2B3 0.06, TBX5-TBX3, CYP1A1, ULK3, FURIN-FES, UMOD, GOSR2, ZNF652, GNAS-EDN3. In children referred for HSCT a trend towards higher BP values was seen. In children assessed 6 months after HSCT more abnormalities in nocturnal BP measurements were seen, which may be a predictor of HT. In children treated with HSCT significant differences in the expression of HT-related genes were found. ABPM was useful in BP monitoring in children treated with HSCT.

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#### P340

##### Hypothyroidism following allogeneic hematopoietic stem cell transplantation for acute myeloid leukaemia

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Hypothyroidism may complicate of allogeneic hematopoietic stem cell transplantation (allo-HSCT); risk factors are analysed. We studied 229 patients with AML who underwent an allo-HSCT between 2003 and 2013 with different conditioning regimens (myeloablative, reduced-intensity, chemotherapy-based, total body irradiation-based). Thyroid stimulating hormone (TSH) and free thyroxin levels (FT4) were available in 104 patients before and after allo-HSCT. Median age at transplantation ( $n=104$ ) was 47 years (IQR 40–59), 37 (35.6%) were female and overall mortality was 34.6% ( $n=36$ ) (Table 1). After a median follow-up period of 47 (IQR 25–84) months, overt hypothyroidism (basal TSH  $> 4.49$  mIU/L, FT4  $< 11.6$  pmol/L) was observed in four patients (3.8%) and subclinical hypothyroidism (basal TSH  $> 4.49$  mIU/L, normal FT4) was observed in 20 patients (19.2%). Positive thyroperoxidase (TPO) antibodies were found in 5 (4.8%) patients. A total of 13 patients (12.5%) were treated with thyroid hormone replacement. Acute graft versus host disease of any grade (aGvHD) occurred in 55 (52.9%) and chronic GvHD of any stage (cGvHD) in 74 (71.2%) of the patients. The risk of developing hypothyroidism was higher in patients with repeated allo-HSCTs ( $P=0.024$ ) and with positive TPO antibodies ( $P=0.045$ ). Furthermore the development of overt hypothyroidism was inversely proportional to age ( $P=0.043$ ). No correlation was found with GvHD, HLA-mismatch and gender. After allo-HSCT a significant number of patients experience thyroid dysfunction including subclinical and overt hypothyroidism. Long-term and continuous follow-up for thyroid function after HSCT is important to provide timely and appropriate treatment.

**Disclosure of conflict of interest:** None.

#### P341

##### Immunomodulatory effects of prophylactic use of ursodeoxycholic acid in allogeneic stem cell transplantation: survival analysis and potential impact on graft versus host disease

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Ursodeoxycholic acid (UDCA) has been shown to have a protective effect in the liver complications after allogeneic stem cell transplantation (allo-SCT), but it also has other immunomodulatory effects; it has been described also a potential benefice as graft-versus-host disease (GVHD) protection. We retrospectively analysed 618 patients consecutively transplanted between 1995–2014 excluding second allo-SCT and those  $< 16$  years old. We analysed the differences between those with and without prophylactic UDCA using SPPS v20. **Results:** the median age was 49 years (16–69) and 59% (362) were males. Other patient characteristics are resumed in Table 1. From 618 patients, 423 (68%) received prophylactic UDCA from the conditioning regimen until day +100, 90% of them after year 2005. With a median follow up of

**Table 1. Baseline characteristics**

	All patients (n=104)	Patients with hypothyroidism (n=24)	Patients without hypothyroidism (n=80)
<b>Baseline characteristics</b>			
Female n (%)	37 (35.6)	9 (37.5)	28 (35)
Age at diagnosis (years), median (IQR)	46 (39-58)	45 (39-57)	47 (39-58)
Age at transplantation (years), median (IQR)	47 (40-59)	46y (40-59)	47 (40-59)
Follow-up period (months), median (IQR)	47 (25-84)	71.5 (39-87)	42 (24-76)
Mortality n (%)	36 (34.6)	5 (20.8)	31 (38.8)
<b>GvHD</b>			
aGvHD n (%)	55 (52.9)	13 (54.2)	42 (52.5)
cGvHD n (%)	74 (71.2)	20 (83.3)	54 (67.5)
Moderate cGvHD n (%)	21 (20.9)	6 (25)	15 (18.8)
Severe cGvHD n (%)	17 (16.3)	5 (20.8)	12 (15)
<b>Hypothyroidism</b>			
TSH before HSCT UI/ml, median (IQR)	1.83 (1.19-2.58)	2.42 (1.85-3.34)	1.56 (1.15-2.29)
fT4 before HSCT mmol/L, median (IQR)	14.6 (13.35-16.75)	14.15 (12.83-16.18)	15.1 (13.4-16.8)
Overt hypothyroidism n (%)	4 (3.85)	4 (16.7)	0
Subclinical hypothyroidism n (%)	20 (19.2)	20 (83.3)	0
TSH max. after HSCT UI/ml, median (IQR)	2.87 (1.88-4.4)	5.58 (4.94-6.97)	2.57 (1.68-3.1)
fT4 after HSCT mmol/L, median (IQR)	14.9 (12.7-16.55)	14 (12.55-14.95)	15.75 (14.25-16.85)
Positive TPO Antibodies n (%)	5 (4.8)	3 (12.5)	2 (2.5)
Therapy with fT4 after HSCT n (%)	13 (12.5)	13 (54.2)	0

39 months (3–179), UDCA administration reduced significantly early (< +100) transplant related mortality (TRM): 16% vs 5% in the univariate ( $P=0.006$ ) and in the multivariate analysis ( $P=0.03$ , HR 0.21; 95% CI 0.05–0.87). It had no influence on overall survival (+2 years estimated: 60% in no-UDCA vs 64% in UDCA group) or overall TRM (+2 years estimated TRM: 23% no-UDCA vs 18% UDCA). UDCA was associated with a lower sinusoidal obstruction syndrome in univariate analysis (3% vs 10%,  $P<0.001$ ), but this observation wasn't confirmed in multivariate analysis, probably due to the statistical power of the deleterious effect of BuCy +/- Flu conditioning regimen in this complication ( $P=0.004$ , HR 10, 95% CI 2.3–47.2) where UDCA wasn't enough to reverse it. Regarding the potential protective effect of UDCA on GVHD, it showed a beneficial effect in hepatic acute GVHD in univariate analysis ( $P=0.048$ ; HR 0.56; 95% CI 0.31–0.99) but these results weren't confirm in

multivariate analysis ( $P=0.14$ ). In addition UDCA-treated group had less extensive chronic GVHD (cGVHD): 62% vs 79%;  $P=0.003$ . When a organ-specific analysis was performed, UDCA was associated with a lower cutaneous ( $P=0.006$ ; HR 0.64; 95% CI 0.46–0.8), lung ( $P=0.001$ ; HR 0.32; 95% CI 0.16–0.62), intestinal ( $P=0.011$ ; HR 0.017; 95% CI 0.38–0.9) and hepatic ( $P=0.002$ ; HR 0.57, 95% CI 0.4–0.82) cGVHD in the univariate analysis (Figure 1) but just lung ( $P=0.01$ , HR 0.26, 95% CI 0.2–0.59) and cutaneous ( $P=0.02$ , HR 0.65, 95% CI 0.45–0.94) maintained significance in multivariate analysis. In fact, UDCA was the only protective factor to prevent lung cGVHD in our series. UDCA-treatment group was associated with less early TRM and less extensive cGVHD, specially lung and cutaneous organ-specific affected. These results must be confirmed in prospective studies and the optimal dose and time of treatment should be defined in future studies.

**Disclosure of conflict of interest:** None.

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<b>Diagnostic</b>		
AML/MDS/MF	288	47%
ALL	73	12%
CLL/NHL/HL	150	25%
MM	53	8%
AA	15	2%
CML	39	6%
<b>Donor</b>		
Sibling HLA identical	391	63%
Sibling 1 or 2 mismatch.	14	2%
Unrelated HLA identical	110	18%
Unrelated 1 or 2 mismatch.	79	13%
Haploidentical	23	4%
<b>Year of allo-SCT 2005-2014</b>	419	68%
<b>Peripheral blood stem cell source</b>	523	85%
<b>Myeloablative regimen</b>	229	37%
<b>GVHD prophylaxis</b>		
CSA + MTX	330	53%
TCR + MTX	77	13%
Calcineurin inh.+ MMF	62	10%
TCR + SIR	136	22%
Others	69	2%
<b>Grade II-IV aGVHD</b>	336	55%
<b>cGVHD</b>		
Limited	322	52%
Extensive	104	17%
	216	41%

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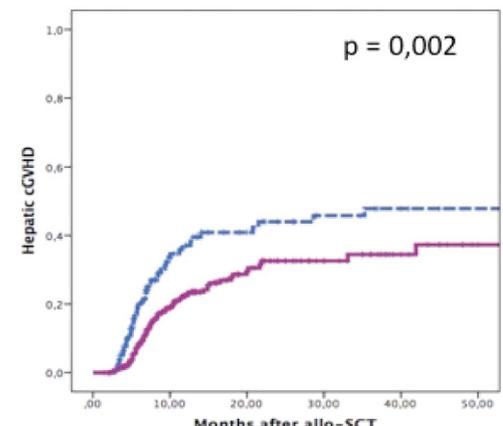
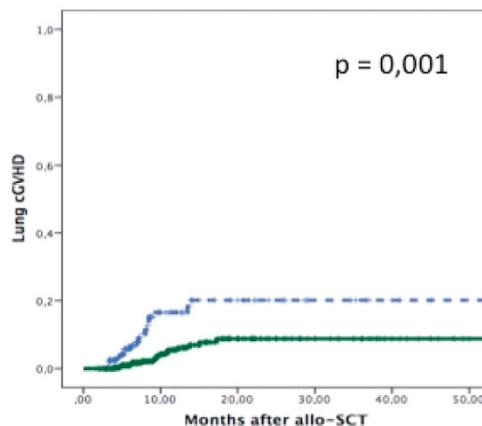
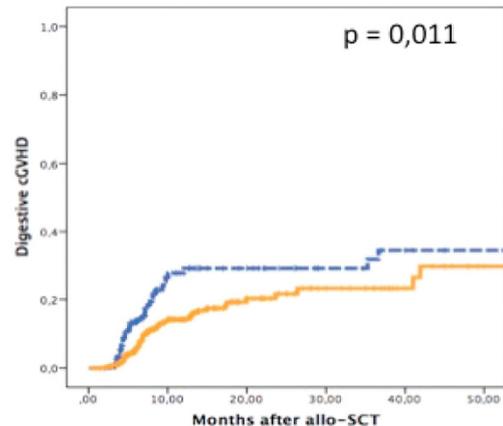
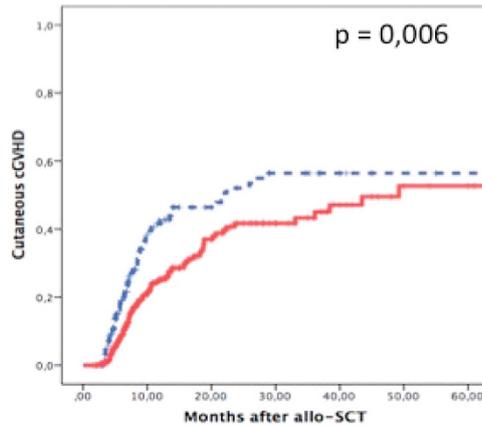
**Impact of disease status on outcomes of allogeneic hematopoietic stem cell transplantation with refractory and relapsed acute lymphoblastic leukemia**

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Objective of study was to evaluate the impact of disease status on the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in the treatment of patients with refractory and relapsed acute lymphoblastic leukemia(ALL). 52 patients with refractory and relapsed ALL, including 19 cases in advanced stage (nonremission, NR) and 33 cases in more than or equal to second complete remission( $\geq$ CR2), received allo-HSCT after myeloablative conditioning regimen in our department. Results: 51 patients engrafted successfully. The transplantation-related mortality (TRM) rate of NR and  $\geq$ CR2 was 10.5% vs 12.1% ( $P=0.815$ ). The incidence of aGVHD was 52.6% vs 57.6% ( $P=0.730$ ), including 42.1% vs 33.3% ( $P=0.527$ ) with mild (grade I-II) and 10.5% vs 24.3% ( $P=0.399$ ) with severe (grade III-IV) aGVHD. The incidence of cGVHD was similar also(41.6% vs 57.9%,  $P=0.660$ ). With a median follow-up of 12(1.8-44.5) months, the cumulative relapse rate of NR and  $\geq$ CR2 was 47% vs 34.3% ( $P=0.425$ ), respectively. The estimated 2 year overall survival (OS) and 2 year leukemia-free survival (LFS) rate were 42.6% vs 45.7% ( $P=0.487$ ) and 46.3% vs 46.2% ( $P=0.571$ ), respectively. Multivariate analysis results showed that cGVHD was independent favorable risk factor for OS and LFS of R/R ALL. For relapsed patients, OS was significantly better with first CR duration >6 months and time to transplant  $\leq$ 2 months. Allo-HSCT is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retrospective analysis showed

Cumulative incidence organ-specific cGVHD.  
 (\*) UDCA-group continuous line; no-UDCA group discontinuous



that R/R ALL with different status prior transplant had similar outcome post transplantation.

**Disclosure of conflict of interest:** None.

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**Previously published**

**P344**

**Impact of previous admission into an intensive care unit on stem cell transplantation outcome**

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The deleterious effect of intensive care unit (ICU) admission during hematopoietic stem cell transplantation (SCT) on patient survival is well established. However, it is unknown whether admission into the ICU during the chemotherapy for

the underlying disease has any impact on survival after SCT. We reviewed patients who had received a first SCT between the years 2000 and 2016 in our institution, and we compared the overall survival (OS), relapse incidence (RI) and non-relapse mortality (NRM) between patients who required ICU admission during the chemotherapy prior to the SCT (ICU group) with matched patients (1:2) who did not need it (NO-ICU group). Sixty-six patients were included, 22 of them in the ICU group and 44 in the NO-ICU group. As shown in Table 1, the main clinic-biologic variables and the SCT procedure were comparable between the patient groups. The causes of ICU admission for the ICU group patients were: 11 (50%) respiratory failure, 4 (18%) septic shock, 4 (18%) neurological disturbance, 2 (9%) post-surgery and 1 (5%) tumor lysis syndrome. Seventeen patients (77%) needed mechanical ventilation. The median time between ICU admission and the SCT procedure was 144 days (range: 106–1097), and the median days of ICU stay were 12.5 (3–57). With a median follow-up after SCT of 5.47 years (0.50–16.22) for the ICU group and 4.52 (0.73–15.85) for the NO-ICU group, the 5 year OS (IC 95%) probabilities were 49% (28–70%) and 45% (29–61%) in the ICU and NO-ICU patients ( $P=0.353$ ), the 5-yr probabilities of relapse were 34% (14–56%) and 42% (25–58%) ( $P=0.755$ ) and the 5-yr probabilities of NRM were 32% (14–52%) and 24% (12–38%) ( $P=0.333$ ), respectively. There were no differences in either OS, RI or NRM between ICU and NO-ICU in the allogeneic or autologous subgroups considered separately. At the moment of the study,

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Variable		ICU before SCT (n=22)	NO ICU before SCT (n=44)	p value
Age	Mean (SD)	38.86 (13.24)	41.82 (14.48)	0.355
	Median [range]	39 [16 - 61]	46 [17 - 64]	0.296
Gender	Male	11 (50%)	21 (48%)	0.862
	Female	11 (50%)	23 (52%)	
Disease	AML/MDS	14 (63%)	28 (63%)	0.985
	ALL	3 (14%)	6 (14%)	
	Lymphoma	2 (9%)	3 (7%)	
	Other	3 (14%)	7 (16%)	
Disease status at SCT	First or second CR	15 (68%)	32 (73%)	0.928
	PR	3 (14%)	5 (11%)	
	Other	4 (18%)	7 (16%)	
Type of SCT	Autologous	7 (32%)	14 (32%)	0.977
	Allogeneic, related	9 (41%)	19 (43%)	
	Allogeneic, unrelated	6 (27%)	11 (25%)	
Time period	2000-2009	8 (36%)	19 (43%)	0.595
	2010-2016	14 (64%)	25 (57%)	
Stem cell source	PB/BM	18 (82%)	41 (93%)	0.161
	UCB	4 (18%)	3 (7%)	
Mismatch	Mismatch	6/15 (40%)	7/30 (23%)	0.207
	No mismatch	9/15 (60%)	23/30 (77%)	
Conditioning therapy	Cyclophosphamide + TBI / Busulfan+Cyclophosphamide	12 (55%)	26 (59%)	0.246
	Fludarabine+Busulfan/ Melphalan+Busulfan	1 (4%)	7 (16%)	
	Melphalan	2 (9%)	5 (11%)	
	Other	7 (32%)	6 (14%)	

TABLE 1. ICU: intensive care unit; SCT: stem cell transplantation; SD: standard deviation; AML/MDS: acute myeloid leukaemia/Myelodysplastic syndrome; ALL: Acute lymphoblastic leukaemia; CR: complete response; PR: Partial response; PB: peripheral blood; BM: bone marrow; UCB: umbilical cord blood; TBI: total body irradiation

12 (54%) of ICU and 22 (50%) of NO-ICU group had died. The causes of death in the ICU group were: relapse in 5 (42%), infection in 4 (33%), GVHD in 1 (8%) and GVHD plus infection in 2 (17%). The causes of death in the NO-ICU group were: relapse in 8 (36.4%), infection in 4 (18.2%), GVHD in 3 (13.6%), GVHD plus infection in 5 (22.7%) and veno-occlusive disease and secondary malignancy, one each (4.55%). In this series, admission to the ICU before SCT did not have an impact on outcomes after SCT. These results suggest that these patients benefit from this treatment as much as the other patients, without expecting worse outcomes as a result of a previous ICU admission. Supported in part by grants RD12/0036/0029 (RTICC, FEJER), P114/01971, Instituto Carlos III, and SGR225 (GRE), Spain.

**Disclosure of conflict of interest:** None.

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#### Improvement of outcome after autologous stem cell transplantation by reduction of infections and SOS/VOD syndrome's related mortality: 28 years single-center experience

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Autologous stem cell transplant (ASCT) is a well established treatment for several haematologic and non haematologic malignancies, either as front-line or rescue therapy. However it

is associated with toxicity and complications which might lead to treatment-related mortality (TRM). Although decrease in TRM has been reported, data about the precise reduction and detailed analysis of causes of mortality throughout years are scanty. The aim of this study was to evaluate early TRM and its causes in patients who underwent an ASCT in a single center along the last three decades. Data of all consecutive adults (> 15 years old) ASCT recipients were prospectively collected at a single Center from December 1988 to August 2016 and then retrospectively analysed. TRM was defined as mortality happened into the 100 days post ASCT or during conditioning treatment due to any cause except relapse or progression of main diagnosis. Demographic characteristics, diagnosis, conditioning regimen and cause of death were analysed. Data were compared for two periods: From December 1988 to December 2000 and from January 2001 to August 2016. A total of 849 patients were included, median age was 45 years (16–71) and 50.3% were male. Diagnoses were: lymphoma (n=391), multiple myeloma (MM) (n=216), acute myeloid leukaemia (AML) (n=93), amyloidosis (AL) (n=15), acute lymphoblastic leukaemia (ALL) (n=39), solid tumours (including breast cancer and germ-cell tumours) (n=89), chronic myeloid leukemia (CML) (n=3), thrombotic thrombocytopenic purpura (TTP) (n=2) and autoimmune disease (n=1). The most frequent indication for ASCT was Lymphoma (46.1%) and MM (25.5%). Twenty patients died within 100 d from ASCT (TRM). Demographic characteristics and causes of death for this patients are shown in Table1. The cumulative incidence of TRM at day +100 was 2.8% (95% CI 1.9–4.1). Comparing both periods, TRM cumulative incidence was 7.9% (95% CI 4.9–11.8) in first period (1988–2000) vs 0.8% (95% CI 0.3–1.8) in last period (2001–2016)  $P < 0.001$ . (Figure 1). According to main diagnosis TRM cumulative incidence was higher in patients diagnosed with solid tumour 6.7% (95% CI 2.7–13.2) and AL 6.7% (95% CI 0.4–26.9) followed by acute leukaemia (AML/ALL) 4.5% (95% CI 1.9–9.1), MM 2.8% (95% CI 1.1–5.6) and lymphoma 1.3% (95% CI 0.5–2.8)  $P < 0.03$  (Figure 2). Sepsis (65%) was the main cause of death in both periods of time, and the only one cause of death in the last period. The second cause was sinusoidal obstruction syndrome (SOS/VOD) (20%), which only appeared in the first period. This study shows a low incidence of TRM in ASCT recipients, with a significant decrease in the last period (2001–2016), despite the higher risk in some groups of patients such as those with amyloidosis and solid tumours. In our experience, infection is the main cause of early death in ASCT recipients and SOS/VOD has disappeared in last years as a cause of early transplant related mortality.

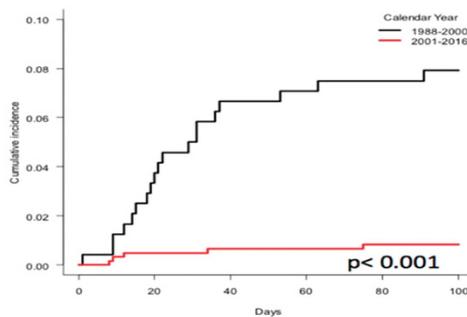
**Disclosure of conflict of interest:** None.

### P346

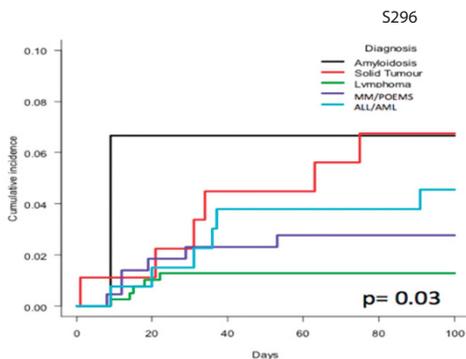
#### Incidence and risk factors for hepatic sinusoidal obstruction syndrome after allogeneic hematopoietic stem cell transplantation: a retrospective multicenter study of Turkish Hematology Research and Education Group (ThREG)

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**Figure 1.** The cumulative incidence at day +100 in both periods of time.



**Figure 2.** Cumulative incidence of TRM depends on the diagnosis



AML: Acute myeloid leukaemia; ALL: Acute Lymphoblastic Leukaemia; MM: Multiple Myeloma; POEMS: POEMS syndrome

Hepatic sinusoidal obstruction syndrome (HSOS) is a potentially life-threatening complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The mean incidence of HSOS was found to be 13.7% (0–40%) in the literature. We examined the incidence and risk factors for HSOS after allo-HSCT. Eight centers from Turkey were enrolled in the study. We retrospectively evaluated the medical records of patients who were treated with allo-SCT between January 2012 and December 2015. A Baltimore criterion was used for assessment of HSOS. Two hundred eighty three (96%) of 295 patients who were treated with prophylaxis with defibrotide alone or one or more of the N-Acetylcysteine, diuretics and heparin used defibrotide (10–25 mg/kg/day). The study included 889 patients (514 males/348 females) with median age of 37 (15–71) years. The demographic and clinical characteristics of patients were summarized in Table 1. The incidence of HSOS was 9.3% (83). Prophylaxis for HSOS was used in 40 (48.1%) of patients, who developed HSOS. Defibrotide as prophylaxis was received by 32 of 40 (80%) of patients. HSOS developed in a median of 13 (0–34) days after stem cell infusion. Seventy-five (90.3%) of patients who developed HSOS had infection at the time of diagnosis. Forty-five of them had ascites, 63 had hepatomegaly and, 74 had weight gain. Seventy-two (86.7%) of patients with HSOS were treated with defibrotide after diagnosis. The median time of starting defibrotide in these patients was 14 (2–29) days. Thirty-six (43.3%) of patients with HSOS recovered completely and forty-seven (56.7%) of them died as a result of multi organ failure. The incidence of HSOS-related mortality in allo-HSCT cohort was found to be 5.3%. In univariate analysis, statistically significant associations were not found between HSOS incidence and age/sex of recipient, type of conditioning regimen, stem cell source and type of GVHD prophylaxis. On the other hand primary diagnosis of myelofibrosis, donor type, engraftment status and prophylaxis for HSOS were significantly associated with HSOS development. HSOS prophylaxis was significantly decreased HSOS-associated mortality ( $P=0.006$ ). HSOS still remains a serious life-threatening complication of allo-SCT. Although the incidence is low, HSOS is associated with increased 100-day non-relapse mortality. HSOS prophylaxis especially with defibrotide, seems to reduce HSOS associated mortality in high risk patients.

[P346]

Table 1: Patients' characteristics

Characteristics	Patients without HSOS (n=806)	Patients with HSOS (n=83)	Statistics
Age, median (range)	38.16±13.53	39.29±12.21	P=NS
Male (n; %)	487 (60.4)	54 (65)	P=NS
Primary diagnosis of myelofibrosis (n; %)	12 (1.49%)	5 (6)	P=0.016
MAC regimen as conditioning (n; %)	438 (54.3)	49 (59)	P=NS
HSOS prophylaxis yes/no (n; %)	255/551 (31.6)	40/43 (48)	P=0.002
MTX/CsA as GVHD prophylaxis (n; %)	756 (93.8)	80 (96.4)	P=NS
Use of PB as stem cell source (n; %)	764 (94.8)	75 (90.4)	P=NS
Use of matched sibling donor (n; %)	684 (84.9)	61 (73.5)	P=0.023
Successful engraftment (n; %)	698 (86.6)	55 (66.3)	P< 0.001

\*Peripheral blood: PB, Bone marrow: BM, MAC: Myeloablative conditioning, NS: not significant, RIC: Reduced-intensity conditioning

Disclosure of conflict of interest: None.

### P347

#### Incidence and risk factors for the development of hemorrhagic cystitis on haploidentical transplantation

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Hemorrhagic cystitis (HC) is a serious complication occurring after allogeneic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10–70%<sup>1</sup> associated mainly with the effect of cytotoxic agents such as Cyclophosphamide (Cy). The conditioning regimen, BKPyV infection and graft versus host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development<sup>2</sup>. With this study we aim to describe the HC incidence and risk factors in all haplo-HSCT performed in the Canary Islands. We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation (PTCy). We used as HC prophylaxis intense hydration on the Cy administration day and the following 24 h (using bladder wash only in 1 patient with cardiac dysfunction) and perfused MESNA at 100% of Cy dose beginning 15 min before the Cy administration on 16 pts and at 20% of the last dose at 0, 4 and 8 h on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC. We performed 20 haplo-HSCT, of which 10 were males (1 was transplanted 3 times) and 8 were women. The mean age was 40 (range: 16–64). The pts presented the following diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). 45% of pts received the haplo-HSCT in remission, 50% with refractory disease and 5% of pts did not receive previous treatment. 6 pts developed HC (36.5% CI at day +80) (Figure 1a) with a median time from haplo-HSCT to onset of 23 days (range: 3–42), 1 (17%) was grade I, 4 (66%) grade II and 1 (17%) grade IV. The grade I case did not receive the MESNA infusion like most of the other pts. No pts died due to HC and all cases resolved without sequelae. 12 pts received Cy pre- and post-transplant and only 8 pts received PTCy. The CI at day +80 for the pts with PTCy was 33.3% and for Cy pre- and post-transplant 38.3% (Figure 1b). We found no statistically significant difference on the CI of HC between these two groups. The development of HC was related to Cy in 1 patient, who suffered from this complication on the second and third haplo-HSCT. For the rest of the pts (after day +30) the HC was related to BKPyV infection, as a consequence of the immunosuppression state of the patient, we also observed all these pts had positive serum viral load for CMV. The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKPyV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker). In our study, HC did not have an impact on mortality of high-risk patients after haplo-HSCT. The HC remains frequent with a high morbidity in particular when it is severe, often causing prolonged hospitalization and resource use. We need further studies to recognize the at-risk population early.

[P347]

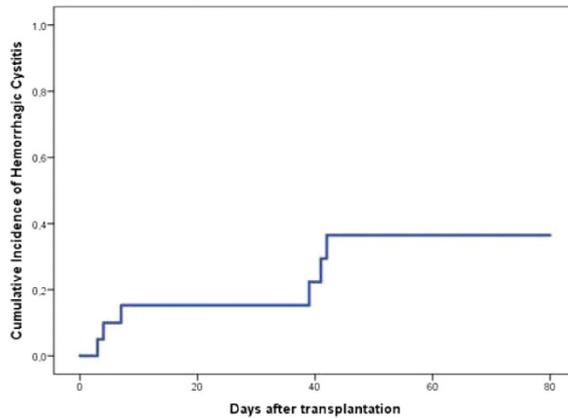


Figure 1a

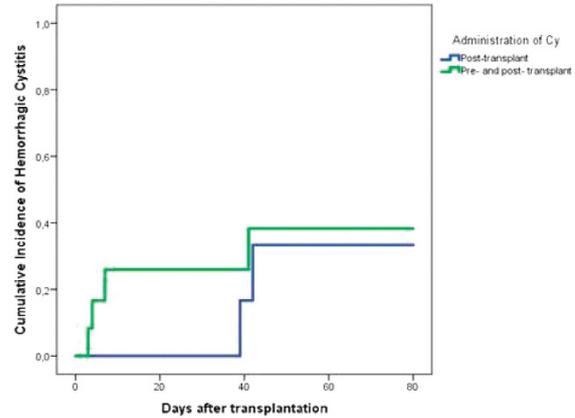


Figure 1b

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Previously published

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### Late onset transplantation-associated thrombotic microangiopathy: a single-centre analysis of incidence and risk factors in allogeneic stem cell transplantation recipients

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Transplantation-associated thrombotic microangiopathy (TA-TAM), with incidence ranging from 0.5 to 76% due to different diagnostic criteria, is a well-known complication after allogeneic hemopoietic stem cell transplantation with 30–45 days median time to onset. Unlike thrombotic thrombocytopenia purpura, the etiology and pathophysiology of TA-TAM have not yet been defined and no standard therapy exists at present. Since few data are available, a single-centre retrospective study was performed to define the incidence and risk factors of TA-TAM in HSCT recipients. From January 2008 to May 2016, 323 allo-HSCT recipients were enrolled in the study. The median age was 45 years (range: 7–70), 156/323 were female; 320 had neoplastic disease (181 AML, 59 ALL, 15 MM, 56 HD/LNH/LLC) and 3 Fanconi anemia. Disease status at transplant was relapse in 88, first complete remission in 157, second complete remission in 66, third remission in 12. 119-/323 patients received matched sibling and unrelated donor transplants, 49 received T cell depleted haplo-HSCT, 145 haplo-HSCT with regulatory and conventional T cell adoptive immunotherapy (T-reg haplo-HSCT) and 10 unmanipulated haplo-HSCT with post-transplant cyclophosphamide. Conditioning regimens included high-dose chemotherapy (cyclophosphamide in 63) with or without TBI (248 and 75 cases, respectively). Most transplant recipients received no post-transplant pharmacological immunosuppression. TA-TAM was diagnosed using the Cho *et al.* diagnostic criteria (2010). In our study 32/323 patients developed TA-TAM. The cumulative incidence was 10% (with relapse and transplant-related mortality as competing risks). Median onset time was 6 months after transplant. TA-TAM was concomitant with GvHD in 3

cases, preceded by an infection in 4 and associated with polisierositis in 8 patients. Multivariate analysis showed no significant relationship with GVHD or infection. Overall mortality was low (2/32), but prognosis was poor due to chronic renal disease in 14/32 patients which was end-stage in 6, severe in 3 and moderate/mild in 5. Two patients underwent kidney transplant. Most cases were observed after T-reg haplo-HSCT (27/145; CI 16%), 4 TA-TAM were observed in 119 recipients of matched sibling or unrelated donor transplants and 1 in 49 haplo T cell depleted HSCT. All 27 cases of TA-TAM in the T-reg haplo-HSCT cohort were associated with TBI vs no-TBI (CI: 26% vs 0%,  $P=0.008$ ). Multivariate analysis confirmed major risk factors were type of transplant (T-Reg haplo-HSCT,  $P=0.06$ ), TBI based conditioning ( $P=0.01$ ), mostly when associated with cyclophosphamide (29% vs 15%,  $P=0.02$ ). TA-TAM is not a rare post-transplant complication and it is potentially fatal. In survivors, it was often associated with long-term morbidity and chronic organ damage, mostly to the kidney with poor renal prognosis. Our retrospective study showed TA-TAM associated risk factors included T reg haplo HSCT as the incidence was highest in this group, TBI-based conditioning or TBI based conditioning plus cyclophosphamide. Although acute GvHD and infection were associated with TA-TAM in retrospective studies, no association emerged between acute GVHD or infection preceding diagnosis in our series of patients. In order to prevent TA-TAM we need to understand its underlying biological mechanism so we are investigating its pathogenesis by means of cytokine assays, histology and murine models.

**Disclosure of conflict of interest:** None.

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### Long-term outcome of ventilated pediatric hematopoietic stem cell transplantation recipients

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Mortality in children requiring invasive mechanical ventilation (IMV) after allogeneic hematopoietic stem cell transplantation (HSCT) is known to be high. Little is known about the long-term outcome of those who survive IMV. We therefore reviewed the medical records of 55 children who survived

IMV after they had received a HSCT between 2000 and 2012 in the two pediatric HSCT centers in the Netherlands. Retrospective multi-center cohort study in two university hospitals that perform all pediatric HSCTs in the Netherlands. Long-term survival of HSCT recipients who had received IMV was assessed. Health status was reviewed more in detail for those who were still alive 2 years after discharge from the pediatric intensive care unit (PICU). In the absence of standardized use of quality of life questionnaires, health status was expressed as the number of affected domains (cardio-respiratory, motor and miscellaneous, regardless of the degree of dysfunction) and level of education. Health status was categorized as follows: no health problems when all four domains were normal; mild health problem when there was an abnormal score in one of the four domains; moderate health problems when there was an abnormal score in two domains; severe health problem when there were abnormal scores in three or all four domains. Between January 2000 and December 2012, 641 patients underwent a HSCT in the two study centers together. A total of 89 HSCT recipients received IMV within 1 year after HSCT (14% of all HSCT recipients). Median time between HSCT and PICU admission was 59 days (IQR 17–102 days). The most common indication to start IMV was respiratory failure (73%). Median duration of IMV was 10 days (IQR 5–18 days). 34 patients (38%) died during their PICU admission. Of the 55 patients who were discharged alive from PICU, 27 patients were still alive 2 years after PICU discharge (49% of those who survived PICU admission). Health status of these long-term survivors was assessed in December 2014 by hospital database review, using the most recent hospital contact. Follow-up time varied from 2 to 11 years (median 6.5 years) after PICU discharge. Two patients (8%) had no health impairment, eight patients (33%) had mild health problems, five patients (21%) had moderate health problems, and nine patients (38%) had severe health related problems. Very little is known about long-term mortality and morbidity of HSCT recipients who survived IMV. Survival of PICU treatment in pediatric HSCT recipients is limited. However, long-term outcome of those who survive PICU treatment seems promising: a considerable proportion of them still is alive 2 years later without obvious sequelae. This is the first study which assessed long-term outcome of IMV after HSCT. Further studies in this population are urgently required to counsel parents and to optimize quality of life outcomes in these children.

**Disclosure of conflict of interest:** None.

### P351

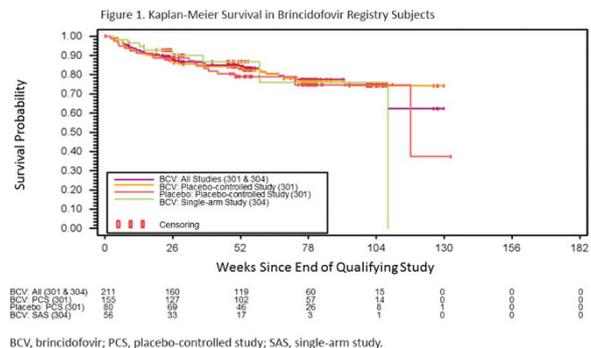
#### Long-term surveillance data support lack of increase in mortality or cancer risk in brincidofovir clinical trial participants

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<sup>1</sup>Chimerix

Brincidofovir (BCV) is an orally bioavailable lipid conjugate of cidofovir (CDV), with broad-spectrum activity against double-stranded DNA viruses, including cytomegalovirus (CMV), adenoviruses (AdV), polyomaviruses (BK and JC viruses), and orthopoxviruses. BCV is being evaluated for prevention of CMV and other DNA viruses in high-risk hematopoietic cell transplant (HCT) recipients, and for the treatment of serious AdV infection. BCV is also being developed for the treatment of smallpox under the US FDA's Animal Efficacy Rule. Because BCV, CDV, and other marketed nucleoside analogs are reported to be carcinogenic in rodents, a Registry was established to evaluate the long-term safety of BCV in subjects who have participated in BCV clinical studies. To date, the Registry includes prior participants from Study 301 (a placebo (PBO)-controlled study of BCV for CMV prevention) and Study 304 (a single-arm study of BCV for AdV treatment). Subjects are encouraged to consent for long-term follow-up in the Registry following participation in BCV clinical studies. Registry

participants are followed at 6-month intervals for a minimum of 3 years from the time of completion of the parent study. Development of malignancies (new or relapsed), life-threatening or fatal adverse events (AEs) assessed as potentially related to BCV, and subjects' vital status are collected. A total of 649 subjects were enrolled in the parent studies (302 BCV and 148 PBO from Study 301, 199 BCV from Study 304). Of these, 291 are enrolled in the Registry as of 24 October 2016 (155 BCV and 80 PBO from Study 301, 56 BCV from Study 304). BCV recipients in the Registry are 60% male, 84% white, with a median age of 47 (< 1–76) years, similar to the BCV recipients in the parent studies. The median duration of follow-up is 12 (0–30) months, with 80% of subjects continuing in follow-up at the time of analysis. All-cause mortality from the time of first dose in the parent study through current Registry follow-up is 25% for BCV vs 22% for PBO ( $P=0.559$ ) in Study 301, and 51% for BCV in Study 304. All-cause mortality in the Registry since completion of the parent study is 21% BCV vs 24% PBO for subjects from Study 301 ( $P=0.618$ ) and 14% BCV for subjects from Study 304 (Figure 1). The incidence of a new malignancy in Registry subjects from Study 301 is 17% BCV vs 23% PBO ( $P=0.295$ ), and the incidence of relapsed primary malignancy is 12% BCV vs 21% PBO ( $P=0.055$ ). In Registry subjects from Study 304, 7% developed a new malignancy and 4% had a relapse of the primary malignancy. No BCV-related life-threatening or fatal AEs have been reported to date in the Registry. Registry data collected to date support no increase in late mortality or increased risk for carcinogenicity in patients treated with BCV. Long-term surveillance for cancer risk and other drivers of mortality is important for novel compounds undergoing development in HCT and other immunocompromised patient populations, with high background risk for these outcomes.

[P351]



**Disclosure of conflict of interest:** All authors of this abstract are employees and stockholders of Chimerix.

### P352

#### Malignancies after long follow-up of pediatric HSCT: a single-centre experience

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Hematopoietic stem cell transplantation (HSCT) is a medical procedure that allows the cure of many paediatric diseases. It has been described an increased risk of new malignancies in this population and it represents an important cause of late mortality. We analyzed the late evolution of 371 patients submitted at pediatric age to hematopoietic transplantation (HSCT) (allogeneic or autologous) in Santa Creu i Sant Pau Hospital between 1984 and 2013. A total of 434 HSCT was analyzed. It has been calculated the cumulative incidence of secondary malignancies at 30 years of follow-up. It has been done univariate and multivariate analysis of risk factors through  $\chi^2$ -test and binary logistic regression method (odds

ratio, OR). It has been studied the relative risk (RR) for new malignancies through comparison of observed cases in our cohort with the expected cases in the general population. We observed 19 cases of secondary malignancies with a cumulative incidence of 6% at 15 years, 12% at 20 years and 36% at 30 years of follow-up. The risk was higher of expected in general population for each tumor type and in the different range of age, being the RR for malignancies in our cohort of 51.4 at 30 years of follow-up. Solid tumors were the most prevalent malignancies (16 out of 19 cases). The median time of latency from HSCT to diagnosis of malignancy was 16 years (1–31 years). The thyroid tumors were the later ones and hematologic malignancies the earliest to be developed. Chronic graft versus host disease was a statistically significant risk factor in univariate (OR=16;  $P=0.006$ ) and multivariate analysis (OR=15.4;  $P=0.000$ ). Total body irradiation of conditioning was a significant risk factor only in multivariate analysis (OR=4.3;  $P=0.03$ ). Previous radiotherapy was a significant risk factor only in univariate analysis (OR=3.1;  $P=0.04$ ). Mortality was 42% (8 out of 19) between patients with a new malignancy and it was the cause of death for all the cases. We observed an incidence of secondary malignancies after HSCT of 5.1% that is significantly higher compared to the expected in the general population ( $P=0.000$ ). The factors that have been related to an increased risk were chronic GvHD, TBI and previous radiotherapy.

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**Disclosure of conflict of interest:** Any competing interest of any the authors must be declared

#### P353

##### Microalbuminuria in long-term survivors of hematopoietic cell transplant: a retrospective single-centre study

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Microalbuminuria defined, as urinary albumin: creatinine ratio (ACR) of 30–300 mg albumin/g creatinine is a marker of endothelial dysfunction and inflammation. In general populations albuminuria predicts development of chronic kidney disease (CKD) and cardiovascular disease (1). In the general population microalbuminuria is associated with the metabolic syndrome (2). In patients with hypertension, diabetes and the critically ill, it is a marker of adverse events and poor outcomes. Following hematopoietic cell transplant (HCT), microalbuminuria at day 100 was associated with a four-fold increased risk of chronic kidney disease (CKD) at 1 year in a single centre study; macroalbuminuria at day 100 was associated with a 6.8-fold increased risk of non-relapse mortality (3). International guidelines for adult and children survivors of HCT recommend that proteinuria is assessed at least annually (4,5). There is a paucity of data on the

prevalence and implications of micro and macroalbuminuria in long-term survivors (> 10 years) of adult HCT, however. This was a single-centre retrospective study conducted in patients attending a dedicated clinic for long-term (minimum 10 years) survivors of HCT. We investigated prevalence of albuminuria and its association with renal disease, cardiac disease and the metabolic syndrome. Of 55 patients, 8 were treated for acute leukemia, 2 for aplastic anaemia and 46 for CML. The median follow up time was 24 years (range: 13–37 years) and the median age at follow up was 55 years (range: 24–81 years). For 36/55 urinalysis was normal (Group A) and 19 (34%) had microalbuminuria (Group B). None had macroalbuminuria. Group B were significantly more likely to have CKD grade 2–4 (eGFR < 60) compared to those in Group A ( $P=0.001$ ). Group B patients were significantly more likely to have diabetes or impaired glucose tolerance 7/19 (37%) vs 2/35 (6%) in group A ( $P=0.005$ ). Group B patients were also significantly more likely to have dyslipidaemia ( $P=0.019$ ) with 14/19 (70%) affected vs 23/35 (37%) in group A. Cardiac disease and hypertension were more frequent in Group B, 4/19 (21%) and 7/19 (37%), respectively vs group A 3/35 (9%) and 8/35 (22%) but these data were not statistically significant. The more features of the metabolic syndrome present, however, (elevated HbA1c, /glucose, dyslipidaemia, hypertension) the more likely a patient was to have microalbuminuria ( $P=0.007$ ). Our data demonstrates that microalbuminuria is a significant finding in long term survivors of HCT. Patients with microalbuminuria are more likely to have CKD grade 2 or below. They are also more likely to have diabetes and dyslipidaemia. As this was a retrospective study we are not in a position to comment on whether microalbuminuria is predictive of the development of renal disease, metabolic syndrome or cardiovascular disease in this group of patients. This warrants further study as intervention, for example with ACE inhibitors, may have the potential to reduce morbidity.

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**Disclosure of conflict of interest:** None.

#### P354

##### Missed and uncontrolled arterial hypertension in long-term survivors after allogeneic hematopoietic stem cell transplantation

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The purpose of the study is the improvement of transplantation techniques and supportive care lead to an increasing number of long-term survivors after allogeneic hematopoietic stem cell transplantation (aHSCT). Recipients of aHSCT have a higher prevalence of cardiovascular risk factors. Ambulatory blood pressure measurement (ABPM) is the 'gold standard' to diagnose arterial hypertension (HT). The prevalence and treatment control of HT by ABPM is unknown in aHSCT patients (pts). This prospective single center study at University Hospital Basel included all pts  $\geq 1$  year after aHSCT in complete hematological remission during annual follow-up consultation. Office blood pressure (oBP) was measured on both arms after 5 minutes rest. ABPM by noninvasive continuous BP monitoring (pulse transit time method) was performed on the same day. HT was defined as oBP  $\geq 140/90$  mm Hg, mean systolic BP  $\geq 130$  mm Hg on ABPM

(BP<sub>24</sub>) and/or current use of antihypertensive drugs. 175 pts (39% female) were included with median age of 53 years (range: 19–75) and 9 years (range: 1–33) after transplantation. 108 (62%) pts received total body irradiation-based conditioning, 70 (40%) pts had chronic graft-versus-host disease, and 39 (22%) required immunosuppression. Mean BMI (kg/m<sup>2</sup>) (±SD) was 25 ± 5, with 22 (13%) pts > 30. Twenty-seven (15.4%) pts were current smokers. Forty-three (25%) pts had chronic kidney disease (eGFR < 60 ml/min/1.73 m<sup>2</sup>) and 17 (10%) diabetes. 82 (47%) pts were on antihypertensive drugs consisting of ACE/AT-II-inhibitors in 55 (31%), calcium-channel blockers in 18 (10%), beta-blockers in 32 (18%) and diuretics in 24 (14%) pts. Thirty-nine (22%) pts were on ≥ 2 drugs. Among our cohort 47 (27%) pts were normotensive without antihypertensive treatment (mean age 46 ± 13 years, 62% female and mean BP<sub>24</sub> (systolic/ diastolic BP) 113 ± 8/76 ± 8 mm Hg). 128 (73%) pts were hypertensive and/or on antihypertensive treatment. Untreated HT was diagnosed in 46 (26%) pts (mean age 52 ± 13 years, 41% female and mean BP<sub>24</sub> of 147 ± 22/91 ± 12 mm Hg), including 14 (8%) with white-coat hypertension and 9 (5%) masked hypertension (normal oBP, high ABPM). In the group of pts with current antihypertensive medication 32/82 (39%) were controlled (mean age 55 ± 13 years, 25% female, and mean BP<sub>24</sub> 119 ± 9/77 ± 7 mm Hg) whereas 50/82 (61%) were hypertensive on ABPM (mean age 55 ± 11 years, 24% female, mean BP<sub>24</sub> 146 ± 13/90 ± 10 mm Hg). Thirty-four (68%) pts with uncontrolled HT were already hypertensive at oBP. Although long-term survivors after aHSCT are known to be at elevated cardiovascular risk, diagnosis of arterial hypertension was missed in every fifth patient. The proportion of controlled hypertension is poor with only 39%.

**Disclosure of conflict of interest:** None.

### P355

#### **Muscle-specific kinase antibody-associated myasthenia gravis post allogeneic stem cell transplantation—successful treatment with rituximab and plasma exchange alone**

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Myasthenia gravis (MG) is a rare complication of allogeneic stem cell transplantation (SCT) and is often associated with graft-versus-host disease (GVHD). We report a 49-year-old man who presented with oculobulbar and neck weakness 30 months after an unrelated donor, allogeneic SCT for chronic myeloid leukaemia (CML). He was diagnosed in 2009 with chronic phase CML. This responded poorly to tyrosine kinase inhibitors (TKIs) and he was found to carry the T315i mutation with additional monosomy 7. He underwent a fully HLA matched unrelated donor SCT with Y<sup>90</sup>-anti CD66 targeted radiotherapy, Fludarabine, Melphalan and Alemtuzumab conditioning. He had grade 1 cutaneous GVHD on ciclosporin withdrawal but no other significant GVHD. He has an immune mediated neutropenia since 4 months post SCT and has reduced immune reconstitution as demonstrated by a sub-normal absolute CD4 level. He remains on Pneumocystis prophylaxis and has not experienced increased infection.

**Treatment and outcome:** he presented with double vision, difficulty swallowing, change in voice character and neck weakness 30 months post SCT. Muscle-Specific Kinase Antibodies (MuSK Ab) were detected confirming Myasthenia gravis. He showed minimal response to Pyridostigmine with progressive swallowing difficulties and dyspnoea. In view of his T315i mutation and Monosomy 7 with poor response to TKIs pre-SCT we elected not to initiate conventional systemic s-

t-

eroid therapy. He commenced weekly plasma exchange (PEX) with Rituximab (100 mg doses) for 8 weeks and then two weekly for 4 weeks to a complete clinical response although he remained MuSK Ab positive. He continued monthly PEX alone for 8 months. His MG symptoms recurred and he received a further 5 doses of Rituximab (200 mg doses) on a monthly basis in association with PEX. He became MuSK Ab negative at the end of this therapy and he has remained negative and symptom free on a monthly PEX maintenance schedule for 15 months. He has remained BCR-ABL negative since transplant.

**Disclosure of conflict of interest:** None.

### P356

#### **Previously published**

### P357

#### **Optimal cooling temperature to prevent adverse effects of chemotherapeutic agents**

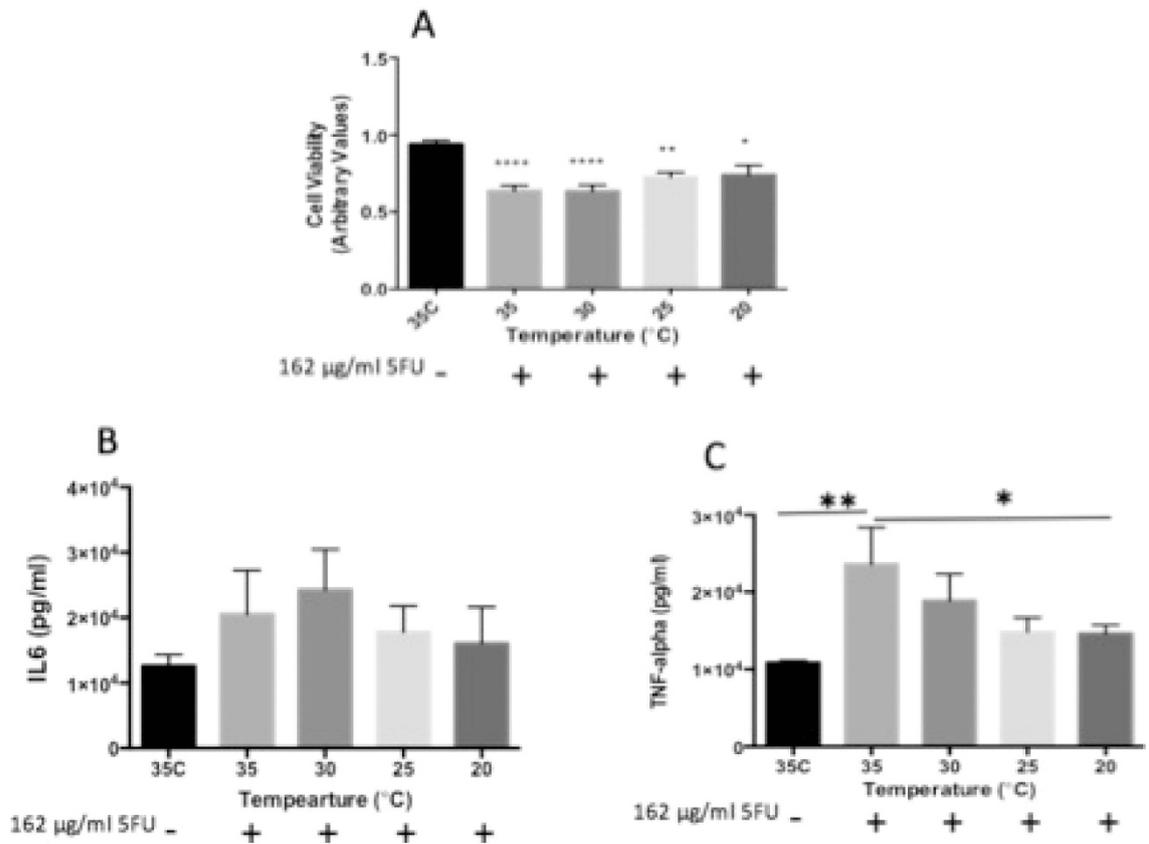
J Walladbegi<sup>1</sup>, A Svanberg<sup>2</sup> and M Jontell<sup>1</sup>

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Chemotherapeutic agents have a cytotoxic effect on the oral mucosa and is a major problem following cancer treatment. Cooling the oral mucosa in conjunction with chemotherapy infusion, using ice chips, is known to reduce the severity of oral mucositis (1, 2). Although effective, ice chips are perceived as uncomfortable. The aim of the present study was to determine the optimal cooling temperature to prevent adverse effect of chemotherapeutic agents using tissue engineered oral mucosal models (TEOM). TEOM were incubated at 35 °C, 30 °C, 25 °C or 20 °C for 30 min followed by exposure to 162 µg/ml of 5-FU for 2 h (control models were incubated at 35 °C). TEOM were then washed and further incubated for 48 h at 37 °C CO<sub>2</sub>. Cell viability and inflammatory cytokine production (IL-6 and TNF-α) were measured using (PrestoBlue) and (ELISA), respectively This study demonstrates an increased capacity to restore cell viability with decreasing temperature (Figure 1a). TEOM treated with 5-FU further showed an increased secretion of the pro-inflammatory cytokines TNF-α and IL-6 at all temperatures compared to un-treated controls. For IL-6, secretion increased markedly when cells were incubated with 162 µg/ml 5-FU at 35 °C and 30 °C compared to cells incubated with medium alone at 35 °C (Figure 1b). For TNF-α, secretion was significantly higher (*P* < 0.05) in cells treated with 162 µg/ml 5FU at 35°C compared to untreated mucosal models and mucosal models treated with 162 µg/ml 5FU but incubated at 20 °C (Figure 1c). TEOM models incubated at 20 °C has an increased capacity to restore cell viability following exposure to 5-FU. Incubation at 20 °C further reduces the release of pro-inflammatory cytokine compared to those incubated at 35 °C.

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**Disclosure of conflict of interest:** This research has been supported by BrainCool AB.

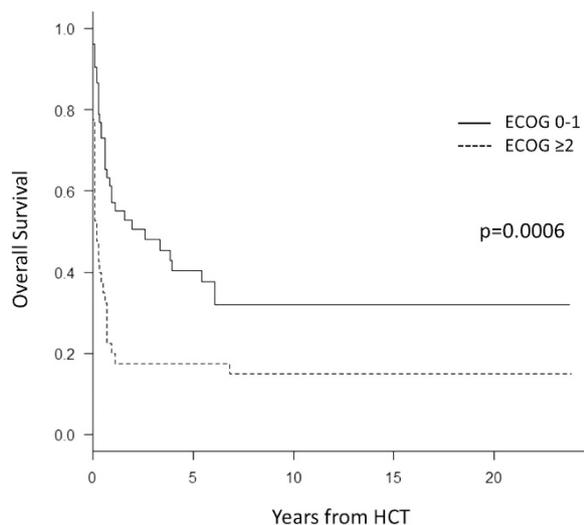
**P358**  
**Outcome following second allogeneic hematopoietic cell transplantation: a single-centre experience**

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Second allogeneic hematopoietic cell transplant (HCT) may be indicated for relapsed haematological malignancies or graft failure. However, there are limited data in the literature concerning outcome post second allogeneic HCT. The purpose of the presented study was to investigate parameters that may influence outcome post second allogeneic HCT. This single centre retrospective study at the Princess Margaret Cancer Centre examined 92 patients that underwent second HCT from 1980 to 2015. First HCT was done for patients with median age 35 years (range: 9–68), for various haematological diseases (AML 26 patients, CML 28 patients, aplastic anaemia 14 patients, ALL 11 patients, other diseases 13 patients). Donors were related for 78 patients (85%), graft was bone marrow for 71 patients (77%). For the second HCT, median age was 40 years (range: 16–69). The indication for second HCT was either relapsed hematologic malignancy for 59 patients (64%, 12 patients relapsed ≤ 6 months post first HCT), vs graft failure for 33 patients (36%). The median time from first HCT until relapse or graft failure was 11 months (1–206 months). The median time from first HCT until second HCT was 18 months (range: 1–212). Year second HCT was performed was grouped into 1980–1995 (n = 28), 1996–2005 (n = 42), 2006–2015 (n = 22).

Fifty-two patients (57%) had ECOG 0–1 vs 40 patients (43%) with ECOG ≥ 2. Preparative regimen was myeloablative (MA) for 47 (51%). Calcineurin inhibitor-based GVHD prophylaxis was used in 75 patients (82%). Eighty-three patients (90%) used the same donor vs only 9 patients (10%) which had a different donor. The type of graft at second HCT was PBSC in 54 patients (59%) vs BM for 38 patients (41%). Among the 82 patients with count recovery data, median days to neutrophil count ≥ 0.5/µL was 18 (range: 8–54), and platelet recovery ≥ 20/µL was 16 days (range: 10–88). Among the 66 patients that died, cause of death was relapse in 17 patients (26%), infection in 18 patients (27%), GVHD in 6 patients (9%), graft failure in 6 patients (9%) and other complications in 19 patients (29%). Median follow up of survivors was 120 months (range: 5–286). Three-year overall survival (OS) of the entire cohort was 35% (95% CI = 25–45). Univariate analysis for OS examined second HCT indication (3-year OS 43% for relapse vs 20% for graft failure, P = 0.02), ECOG score (3-year OS 48% for ECOG 0–1 vs 18% for ECOG ≥ 2, P = 0.0006, see Figure), time from first HCT to relapse/graft failure (3-year OS for < 12 months 21% vs ≥ 12 months 46%, P = 0.009), preparative regimens (3-year OS for MA 42% vs other regimens 23%, P = 0.08). Age at second HCT (≤ 40 vs > 40 years, P = 0.2), time period second HCT was performed (P = 0.7), use of different donor (P = 0.3) and graft source for second HCT (P = 1.0) did not significantly influence OS. Multivariable analysis for OS demonstrated ECOG score at second HCT (HR = 2.15 for ECOG ≥ 2, 95% CI = 1.32–3.51, P = 0.002) and indication for second HCT (HR = 1.67 for graft failure, 95% CI = 1.02–2.75, P = 0.04) to be the only independent prognostic variables influencing survival in this cohort. Second HCT may provide prolonged survival, particularly for patients transplanted for relapsed disease following first HCT, and with a favourable performance status.

[P358]



**Disclosure of conflict of interest:** None.

**P359**

**Outcomes of allogeneic stem cell transplantation (alloSCT) for myeloid malignancies in patients over 60 years of age: a single-centre experience**

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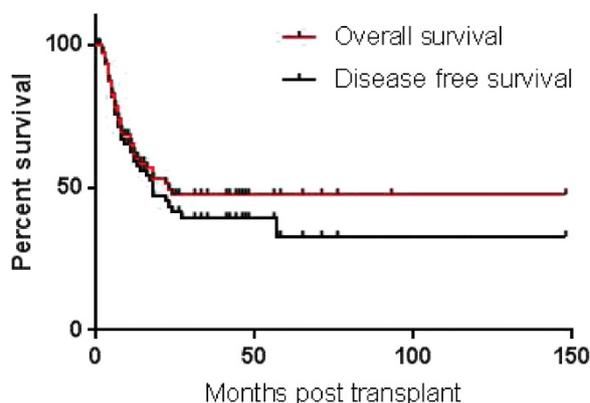
Older age has been implicated as a negative predictor of survival after allogeneic stem cell transplantation, the presence of comorbidities and poor physical reserve negatively impacting on survival. We present the outcome of AlloSCT in 76 patients, aged between 60 and 74 years, who underwent transplantation for myeloid malignancies in the Wessex Blood and Marrow Transplant Unit, Southampton UK. Patients 76 patients over the age of sixty underwent first AlloSCT between August 2004 and September 2016 for poor risk myeloid malignancies; AML (32), secondary AML (24), myelodysplastic syndrome (18) and CMML (2). Indications for AlloSCT in the AML group included the presence of FLT-3 ITD (8), poor risk cytogenetics (3), primary refractory disease (4), relapsed refractory disease (5) and high risk disease (12). The cohort included 56 male and 20 female patients, median age 64 years (range: 60–74). Stem cell sources were from HLA matched sibling (10), matched unrelated (45) and single antigen mismatched unrelated (21) donors. CMV status: 61 transplants were CMV matched (24 -/-, 37 +/-) and 15 mismatched (6 -/+ , 9 +/-). All patients received reduced intensity conditioning; fludarabine/busulfan (Flu/Bu) (47), flu/bu/clofarabine (5), flu/melphalan (flu/mel) (16), flu/mel and targeted radiotherapy (2) and one received fludarabine and cyclophosphamide. All patients received Campath-1H as part of the conditioning regimen. Stem cell source: peripheral blood stem cells 73 patients and 3 BM. Comorbidity was assessed using the haematopoietic cell transplantation co-morbidity index (HCT-CI), with 16 patients (21%) having no co-morbidities, 35 (46%) a co-morbidity index of 1–3 and 25 (33%) had a score  $\geq 4$ . Follow up of survivors ranged from 1 to 148 months (median: 32 months). At the specified end point 29 patients had relapsed (38%) with an actuarial 3-year relapse rate of 55%. There were 34 deaths (44%). Relapse (23) was the main cause of death with transplant related mortality of 5% (4) at day 100, 8% (6) at 6 months and 13% (10) at 1 year. The actuarial OS at 3 years was 48%, with a 3-year DFS of 39%. Of the surviving

relapsed patients all received chemotherapy and donor lymphocyte infusions resulting in effective recovery of remission, showing the utility of this approach. In terms of co-morbidity, actuarial survival rates were 60% in those with an HCT-CI index of 0, 39% with an index of 1–3 and 50% with an index  $\geq 4$ . The results of this retrospective study indicate that AlloSCT using reduced intensity conditioning regimens can be an effective treatment strategy for older patients with high risk myeloid malignancies including those with significant co-morbidities. Relapse remains the main cause of treatment failure and strategies to reduce relapse risk are required. Patients that relapse post AlloSCT may respond to further treatment such as azacytidine or intensive chemotherapy and donor lymphocyte infusions.

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[P359]



**Disclosure of conflict of interest:** None.

**P360**

**Patients and clinicians communication: do we agree when we talk about quality of life?**

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Effective patient-centered communication is associated with health outcomes, such as symptom management and quality of life (QoL). However, studies assessing communication in the hematopoietic cell transplant (HCT) field are scarce. Thus, we assessed (1) clinicians' agreement on patient-reported QoL during the early post-transplant period, (2) potential direction of disagreement (under- vs over-estimation), and (3) whether patient-related variables were associated with disagreement. This is a secondary analysis of a cross-sectional multicenter study where patients and clinicians completed an identical QoL questionnaire (FACT-BMT) at day 90. Clinical and demographic variables as well as anxiety and depression (HADS) were collected. Agreement was analyzed with the intraclass coefficient correlation (ICC). Rates of under- and over-estimation were calculated. Logistic regression models identified predictors of disagreement. We analyzed 96 pairs of

questionnaires, filled in by 96 patients and 11 clinicians. Patients' median age was 54 years, 50 (52%) were men, and 50 (52%) received an allogeneic HCT. Clinicians' median age was 42 years, 7 were men and had worked on the transplant field for a median of 12 years (range: 3–23). Agreement on QoL was moderate (ICC=436). Exploratory analyses revealed that agreement for emotional (ICC=092) and social (ICC=270) wellbeing was poor, whereas it was moderate for physical (ICC=457), functional (ICC=451) and BMT Concerns (ICC=445). Patients' wellbeing was overestimated in 41–59% of the categories of wellbeing parameters, and underestimated in 10–24%. Patient-related variables explained 12–17% of the variance on disagreement across scales. Specifically, anxiety contributed to disagreement in all subscales, except in social wellbeing, where non-significant univariate associations were observed ( $P>0.05$ ). Type of transplant (allogeneic vs autologous), performance status, and graft-versus-host disease were not associated with disagreement ( $P>0.05$ ). Patients and clinicians agreement on QoL is suboptimal, particularly on emotional and social wellbeing. Patients' wellbeing cannot be estimated from other sources than themselves. These results highlight the unmet needs of HCT recipients with respect to QoL-related issues; an outcome that must be addressed by HCT programs since their wellbeing is as important as survival endpoints.

**Disclosure of conflict of interest:** None.

### P361

#### Previously published

### P362

#### **PICC (peripherally inserted central catheter) is a valid device alternative to standard central venous catheter in patients submitted to autologous stem cell transplantation**

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PICC (peripherally inserted central catheter) is known to be less traumatic and with lower risk in the positioning phase compared to a standard central venous catheter (CVC). We wanted to test the function and the safety of PICC device as alternative to standard CVC in patients submitted to autologous stem cell transplantation (ABMT). The primary end point of the study was to individuate the cause leading to the failure of PICC (its removal or the need of another CVC during the ABMT procedure). Secondary end points were the correct function of the device and its praticity. Twenty patients submitted to ABMT for multiple myeloma (18) or lymphoma (2) experienced a double lumen PICC device (17) or a single lumen (3) if the patient already carried a permanent single lumen CVC such as Hickman or Port-a-Cath. We excluded from this experience patients with high risk of life-threatening situation or high risk of intensive care already before ABMT. PICC devices were placed from a specialistic nurse team by ultrasound identification of a deep venous vessel in upper arms. Melphalan 200 or CEAM were the standard conditioning regimens employed in myeloma and lymphoma ABMT respectively. We considered a failure all the causes leading to the removal of PICC or requiring another CVC before the end of the transplant procedure. At last we collected nurses and clinicians opinions about the PICC functionality. No complication has been recorded in positioning phase. 19/20 patients maintained the PICC device for all the time of transplant procedure. Only one patient needed to remove the device for infection. The opinion of nurses and clinicians about the PICC device was a significantly slower speed of infusion and resistance to the flow; in fact, 11/20 patients needed an

infusional pump. The idraulic resistance of the catheter was particular evident against cellular fluids (stem cells suspension, transfusions of blood and platelets). For this reason PICC seems to be less indicated in patients requiring many endovenous infusions (nurses' opinion). The rate of infection of PICC devices seems to be lower compared to CVC, but the number of cases tested in this experience is too limited for definitive conclusions about it. For other aspects PICC is similar to other CVCs. PICC seems to be a valid alternative to standard CVC in patients who do not require intensive care, and in particular in patients with low intensity ABMT who do not present a high number of endovenous infusions. Maybe PICC is less burdened of infections respect to normal CVC. This fact, summed to the lower risk during the positioning of the device, leads to consider the use of this device in ABMT setting for standard risk patients.

**Disclosure of conflict of interest:** None.

### P363

#### **Platelet transfusion refractoriness after T-cell-replete haploidentical transplantation is associated with inferior clinical outcomes**

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Haploidentical stem cell transplantation (haplo-SCT) has been an alternative source of bone marrow for patients without human leukocyte antigen (HLA)-matched donors. The aim of this study was to investigate the relationships between platelet transfusion refractoriness (PTR) and clinical outcomes in the setting of haplo-SCT. Between May 2012 and March 2014, 345 patients who underwent unmanipulated haplo-SCT were retrospectively enrolled. The log-rank test and Cox regression models were used to determine the impact of PTR on clinical outcomes. PTR occurred in 20.6% of all patients. Patients in the PTR group experienced higher transplant-related mortality (TRM, 43.7% vs 13.5%,  $P<0.001$ ), lower overall survival (OS, 47.9% vs 76.3%,  $P<0.001$ ) and lower leukemia-free survival (LFS, 47.9% vs 72.3%,  $P<0.001$ ) compared to those of patients in the non-PTR group. The multivariate analysis showed that PTR was associated with TRM ( $P=0.002$ ), LFS ( $P<0.001$ ), and OS ( $P<0.001$ ). The cumulative incidences of PTR in patients receiving  $>12$  PLT transfusions (third quartile of PLT transfusions) were higher than in patients receiving either  $>6$  (second quartile) or  $>3$  (first quartile) PLT transfusions (56.1% vs 41.6% vs 28.2%, respectively;  $P<0.001$ ). The multivariate analysis also showed that PTR was associated with the number of PLT transfusions ( $P<0.001$ ). PTR could predict poor transplant outcomes in patients who underwent haploidentical SCT.

**Disclosure of conflict of interest:** None.

### P364

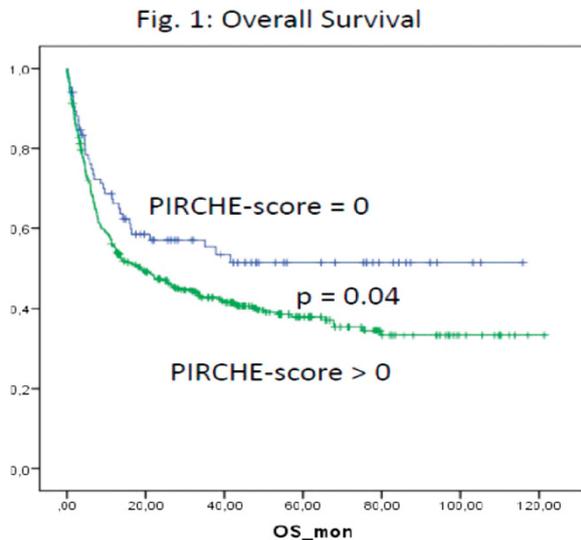
#### **Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) are associated with poorer outcome after single mismatch unrelated donor stem cell transplantation: a study of the German Cooperative Transplant Study Group (GCTSG) within the German working group for bone marrow and blood stem cell transplantation (DAG-KBT)**

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Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf

There are only few algorithms for the selection of HLA-mismatched unrelated donors, when no fully matched donor is available. Indirect recognition of HLA-mismatches can be predicted using the model of 'Predicted Indirectly ReCognizable HLA Epitopes' (PIRCHE). The PIRCHE model is a recently

developed computer-based strategy, which classifies HLA-derived epitopes that are potentially presented by patient-donor shared HLA-molecules. We performed a multicenter retrospective study evaluating the impact of PIRCHE on outcome after allogeneic stem cell transplantation from HLA 9/10 matched unrelated donors. The study cohort included 1997 adult patients who had undergone allogeneic stem cell transplantation for AML or MDS. PIRCHE scores were computed for 424 recipients of HLA 9/10 matched unrelated donor transplants (9/10MUD) using a web-based tool. Primary Endpoint was overall survival at 2 years. Patients with a 9/10 MUD were divided into 2 groups according to the sum of PIRCHE I+II values (PIRCHE score). Eighty-five (85) patients had a PIRCHE score of 0 (no PIRCHE detected), 339 a PIRCHE score >0. KM estimate of 2 year OS was higher for 9/10 MUD with PIRCHE score=0 compared to PIRCHE score >0: 57% (95% CI: 51–63%) vs 47% (95% CI 41–53%),  $P=0.04$ . OS was similar for 9/10 MUD with PIRCHE score=0 and 10/10 MUD (57% vs 55%). Cox regression analysis revealed poorer OS for PIRCHE scores >0 (RR 1.5, 95% CI: 1.0–2.1,  $P=0.03$ ). Cumulative incidence of NRM at 2 years was lower for 9/10 MUD with PIRCHE score=0 compared to PIRCHE score >0 (20% vs 32%,  $P=0.05$ ). Multivariate Cox regression analysis revealed poorer NRM for PIRCHE score >0 (RR 1.7, 95% CI: 1.0–2.9,  $P=0.03$ ). Cumulative incidence of aGVHD grade 2–4 at 6 months was not significantly different for 9/10 MUD with PIRCHE score 0 compared to PIRCHE score >0 (23% vs 30%,  $P=0.2$ ). Cumulative incidence of cGVHD at 2 years was lower for 9/10 MUD with PIRCHE score 0 compared to PIRCHE score >0 (31% vs 49%,  $P=0.04$ ). Our findings require confirmation, ideally in a large prospective cohort study. If validated, the PIRCHE model would allow selection of permissible HLA-mismatches that may be associated with an improved transplant outcome in terms of reduced NRM and better OS.

[P364]



**Disclosure of conflict of interest:** None. This study was supported by a research grant from PIRCHE-AG to the University Medical Center, Hamburg-Eppendorf.

**P365**

**Pre-transplant evaluation of liver dysfunction including elastography in patients receiving allogeneic hematopoietic cell transplantation**

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Pretransplant liver dysfunction has been recognized as a risk factor for complications and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). However, there is no consensus on the optimal way to evaluate liver function in HCT candidates. Transient elastography (TE) is a non-invasive method for diagnosing liver damage and cirrhosis. While elastography is widely used in the setting of viral hepatitis, its possible role in allo-HCT recipients has not been deeply evaluated. Patients receiving allo-HCT in our center from May 2014 are scheduled to receive pretransplant evaluation by a hepatologist under a prospective protocol. The evaluation includes a hepatologist consultation, liver function and infectious serology tests and TE. All patients receive ursodiol from HCT admission to day +30. This study constitutes the first evaluation of the ongoing protocol for patients receiving their first allo-HCT from May 2014 to August 2016. Sixty patients received a first allo-HCT during the study period. Sixteen patients did not undergo hepatologist evaluation due to timing issues ( $n=6$ ), unstable medical condition ( $n=4$ ) or other reasons ( $n=6$ ). Finally, 44 patients received pretransplant evaluation by a hepatologist under the current protocol and constitute the study population. Median age at transplantation was 51 years (range: 21–69). Most patients received a transplant for acute leukemia ( $n=23$ , 52%) or non-Hodgkin's lymphoma ( $n=10$ , 23%) mainly from HLA matched unrelated donors ( $n=21$ , 48%). Thirty-two patients received reduced-toxicity regimens (73%). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus in combination with another agent. Median follow-up for survivors of 14 months (range: 3–29). Median elastography was 5.6 kPa (range: 2.9–13.7). Considering the HCT-CI categories on hepatic dysfunction, 38, 6 and 0 patients scored 0, 1 and 3 points, respectively. There were two cases of veno-occlusive disease (VOD). Overall survival and non-relapse mortality of all patients at median follow-up were 76% (95% CI 69–83) and 22% (95% CI 14–30), respectively. In the univariate analysis, median elastography was not associated with a higher risk of NRM ( $P$ -value=0.13), OS ( $P$ -value=0.11) or hepatic chronic GVHD ( $P$ -value=0.32). The two patients with VOD had normal pre-HCT transaminase levels and TE. This first analysis of an ongoing protocol with universal pre-HCT evaluation of hepatic function indicates that increased values of transient elastography are not associated with higher NRM or lower OS after the procedure. Further studies including a larger number of patients are needed in order to clarify the possible role of elastography in the HCT setting.

**Disclosure of conflict of interest:** None.

**P366**

**Pretransplant lung function and performance status as mortality predictors after allogeneic hematopoietic stem cell transplantation: a single-center cohort study**

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Allogeneic hematopoietic stem cell transplantation (HSCT) remains associated with a high morbidity and mortality in spite of advances in HSCT management. Specifically, pulmonary complications account for a substantial proportion of deaths within the first 100 days after HSCT. Therefore, identification of lung dysfunction and additional comorbidities are crucial for preventive strategies in HSCT. Given the inconsistent association of pretransplant lung function

parameters on mortality after HSCT and the significant changes in HSCT care over the last decades, the aim of our study was to assess the effect of pulmonary function and comorbid conditions on mortality in patients undergoing HSCT for hematological disorders. We retrieved relevant clinical data of all consecutive patients at the Hematology division of the Basel University Hospital with a transplant for hematological disorders between 2008 and 2015. We examined the lung function at baseline and 3, 6 and 12 months after HSCT—including the 1 s forced expiratory volume (FEV1% of predicted), FEV1/VCmax and diffusing capacity for carbon monoxide (DLCO, adjusted for hemoglobin concentration). In addition, we assessed pretransplant conditions such as age, sex, Karnofsky performance status (KPS), donor type and various risk scores in HSCT (hematopoietic cell transplantation comorbidity index (HCT-CI), European Society for Blood and Marrow Transplantation (EBMT), revised pretransplant assessment of mortality score (PAM)). Using uni- and multivariate Cox proportional-hazards regression analysis, we evaluated patient- and transplant-related risk factors for all-cause mortality by including the following categorical candidate variables: FEV1 ( $\geq 80\%$  vs  $50\text{--}79\%$  vs  $< 50\%$  of predicted), KPS ( $< 90\%$  vs  $\geq 90\%$ ), age ( $< 54$  vs  $\geq 54$  years), conditioning intensity and donor type (matched-related vs mismatched-related vs matched-unrelated vs mismatched-unrelated). Within the study period, 429 patients with predominantly acute leukemia (64%) or lymphoproliferative disorders (28%) underwent myeloablative ( $n=330$ ) and non-myeloablative ( $n=99$ ) HSCT at a median age of 54 years (range: 19–72 years). The analysis of the HCT-CI, KPS, EBMT and PAM score revealed median values of 2 (range: 0–8), 90% (range: 30–100%), 4 (range: 1–7) and 15 (range: 0–35), respectively. In univariate and multivariate analyses with a median follow-up of 12 months (interquartile range, 3–36 months), a FEV1 of  $50\text{--}79\%$  vs particular lower FEV1 of  $< 50\%$  and an impaired KPS  $< 90\%$  was significantly associated with a higher risk for all-cause death (univariate hazard ratio (HR), 1.7; 95% confidence interval (CI), 1.2–2.5;  $P=0.006$  vs HR 2.8; 95% CI, 1.4–5.8;  $P=0.004$ ; and HR 1.8; 95% CI, 1.3–2.5;  $P=0.001$ ; and multivariate HR 1.6, 95% CI, 1.1–2.4;  $P=0.011$  vs 2.5; 95% CI, 1.2–5.1;  $P=0.014$ ; and multivariate HR 1.7; 95% CI; 1.2–2.4;  $P=0.002$ )—independent of age, conditioning regimens and donor type. Taken into account, the changes in practices, supportive care and management of comorbidities, in our cohort, a reduced pretransplant lung function and impaired performance status remain independent predictors of mortality in HSCT. In line with our results, further analysis should focus on measures of function, disability, comorbidity, frailty and mental health to provide a basis for interventions further reducing morbidity and mortality in HSCT.

**Disclosure of conflict of interest:** None.

### P367

#### Prophylactic recombinant thrombomodulin prevents hepatic veno-occlusive disease/sinusoidal obstruction syndrome in high-risk pediatric patients that undergo hematopoietic stem cell transplants

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Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning for hematopoietic stem cell transplantations (HSCT). Recombinant thrombomodulin (rTM) is a new drug for treating disseminated intravascular coagulation (DIC) and is an endothelial anticoagulant cofactor that promotes the thrombin-mediated formation of activated protein C (APC). rTM has been used to treat VOD/SOS, but its ability to prevent VOD/SOS has not been established. We evaluated the cases of 19 pediatric hematology and oncology patients (8 (43%) acute myeloid leukemia, 3 (16%) acute lymphoblastic leukemia, and

4 (21%) neuroblastoma patients, and 1 (5%) patient each with myelodysplastic syndrome, rhabdomyosarcoma, hemophagocytic syndrome (HLH), and Wiskott–Aldrich syndrome) who underwent HSCT at our institution between 2007 and 2014 and had  $\geq 1$  risk factors for VOD/SOS. These risk factors included previous treatment with gemtuzumab ozogamicin (GO), receiving  $> 2$  HSCT, undergoing conditioning with busulfan (BU), and being diagnosed with HLH. The patients who received HSCT after 2012 ( $n=8$ ; rTM group) were treated with rTM as a prophylaxis against VOD/SOS (380 U/kg per day for 7 days; from days 7 to 13) together with ursodeoxycholic acid (urso) and low-molecular-weight heparin (LMWH), and the others ( $n=11$ ; control group) were only treated with urso and LMWH. The incidence of VOD/SOS was evaluated, and various coagulation parameters and markers of endothelial injury (plasminogen activator inhibitor type (PAI-1) and APC) were measured in both groups. The patients' median age was 2 (range: 0–18) years, and 11 (58%) were male. Clinical characteristics, including VOD/SOS risk factors, were well-matched in both groups. The risk factors possessed by the patients included receiving  $> 2$  HSCT (9/19, 47%), previous GO treatment (6/19, 32%), conditioning with BU (3/19, 16%), and a diagnosis of HLH (1/19, 5%). Although VOD/SOS occurred by post-HSCT day +35 in 3 (27%) patients in the control group, VOD/SOS was not seen in the rTM group. Two of the former 3 patients (2: previous treatment with GO, 1: a diagnosis of HLH) suffered severe VOD/SOS, and 1 (a diagnosis of HLH) died of the condition. No grade 3/4 adverse events involving bleeding or severe organ damage were reported in the rTM group. Interestingly, the mean peak value of PAI-1 and APC (markers of endothelial injury) were significantly lower in the rTM group (Table 1).

### [P367]

Table 1

	rTM (n=8)	Control (n=11)	p-value
VOD/SOS incidence	0 (0%)	3 (27.3%)	0.228
Coagulation tests			
D-dimer ( $\mu\text{g/ml}$ )	$2.9 \pm 2.2$	$8.3 \pm 11.2$	0.2
PT-INR	$1.13 \pm 0.11$	$1.31 \pm 0.54$	0.36
Fibrinogen (mg/dl)	$506.9 \pm 154.1$	$627 \pm 141.9$	0.09
PAI-1	$24.1 \pm 10.6$	$92.2 \pm 87.5$	0.04
APC (%)	$67.8 \pm 11.9$	$41.9 \pm 23.7$	0.01

The present findings suggested that prophylactic rTM after HSCT might help to prevent VOD/SOS. It is considered that rTM possesses protective activity against endothelial damage. Further prospective studies are required to determine the efficacy and optimal duration and dosage of prophylactic rTM treatment since there is no consensus about how rTM should be used as a prophylactic treatment against VOD/SOS.

**Disclosure of conflict of interest:** None.

### P368

#### Protective effect of early human cytomegalovirus reactivation on relapse of myeloproliferative disorders after allogeneic hematopoietic stem cell transplantation

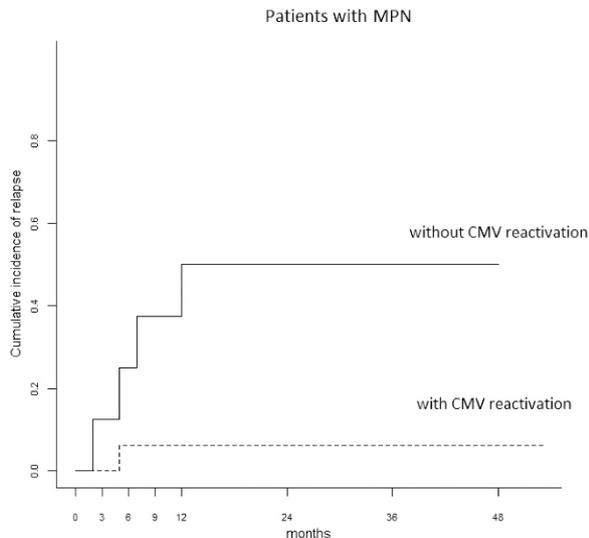
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There have been conflicting results regarding the association between early cytomegalovirus (CMV) reactivation and decreased incidence of relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). This prompted us to retrospectively evaluate the potential impact of CMV reactivation on transplantation outcomes in a study population of 161 consecutive adult patients who underwent allo-HSCT in our institution and were treated and followed in a homogenous manner. Patients were monitored for CMV reactivation once weekly for the first 100 days after allo-HSCT. Monitoring was done with a real time qPCR with lower limit of detection of 150 genome copies per ml of blood. When CMV viremia was detected, all patients were treated with intravenous ganciclovir or oral valganciclovir until two consecutive negative qPCR assays. Univariate and multivariable proportional hazards

models using the Fine and Gray approach were considered to evaluate the variables for relapse, treating death as competing event. Between 2011 and 2014, 97 male and 64 female patients underwent allo-HSCT at a median age of 46 years (range: 18–64). Among them, most patients were treated for myeloid malignancies (74 AML, 11 MDS and 24 MPN with 11 CML, 11 MF and 2 CMML), while the rest had lymphoproliferative disorders (24 ALL, 10 NHL, 6 MM, 5 MH and 6 CLL) and one patient had aplastic anemia. The donors were unrelated in 79 cases, related in 77 patients and haploidentical in 5 patients. Most of the patients (70%) received peripheral blood stem cells after a reduced-intensity conditioning regimen (56%). With a median follow-up of 23 months, early CMV reactivation occurred in 62% patients at a median of 27 days after transplantation and did not affect relapse incidence in patients with lymphoproliferative disorders. On the contrary, the cumulative incidence (CI) of hematologic relapse in patients with myeloproliferative disorders (AML and MPN) at 20 months after allo-HSCT was 36% (95% CI, 21–52%) in patients without, opposed to 18% (95% CI, 10–29%) in patients with CMV reactivation ( $P=0.04$ ). However, CMV reactivation did not significantly affect ( $P=0.21$ ) overall survival between patients with (64%; 95% CI 53–77%) and without CMV reactivation (48%, 95% CI 34–68%). A striking and previously unreported correlation between CMV reactivation and relapse was found in patients with MPN; the CI of relapse was 50% (95% CI, 12–80%) in patients without, opposed to only 6% (95% CI, 25–100%) in patients with CMV reactivation ( $P=0.01$ ). A substantial and independent reduction of the relapse risk in myeloproliferative disorders (AML+MPN) associated with early CMV reactivation was confirmed by multivariate analysis using time-dependent covariate functions for high-risk disease, use of ATG, chronic graft-versus-host disease (hazard ratio 3.33; 95% CI, 1.09–10.09,  $P=0.03$ ), and CMV reactivation (hazard ratio 2.37; 95% CI, 1.05–5.37,  $P=0.04$ ). In summary, this report supports an independent role of CMV reactivation on relapse in patients with myeloproliferative disorders. To our knowledge, we are the first to show a significant reduction of relapse incidence in patients with MPN, even though our findings are based on a relatively small number of patients. However, this putative virus-versus-myeloproliferation effect definitely warrants further research.

[P368]



**Disclosure of conflict of interest:** None.

### P369

#### Quality of life and donor–recipient relationship after HLA-matched sibling hematopoietic stem cell transplantation—methods and preliminary results

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Despite the fact that the choice of donors and the sources of hematopoietic stem cells have enlarged, preferred donor for allogeneic hematopoietic stem cell transplantation (HSCT) remain HLA-matched sibling (MSD). Transplant donation between sibling is an unique life experience that may have an impact on their future relationship. The aim of the study was (1) to quantitatively measure the quality of life (QoL) in transplanted patients and (2) to qualitatively describe the relationship between recipient and sibling donor after MSD-HSCT. We invited to participate in this survey 82 adults aged 18.0–38.7 years (median: 23.6) who underwent MSD-HSCT in our centre and their sibling donors. Forty-five subjects (54.9%) and their siblings consented to take part in the study and returned the questionnaires. Finally, the studied group consisted of 45 MSD-HSCT recipients (W:M=19:26) aged 18.0–36.2 (median: 28.5) years, who underwent MSD-HSCT at age of 5.8–16.3 (median: 11.9) years and their sibling donors aged 21.0–36.0 (median: 31.0) years, who were at age of 11.2–20.2 (median: 15.5) years at bone marrow harvesting. For the quantitative measurement of QoL, the fourth version of the questionnaire functional assessment of chronic illness therapy–bone marrow transplantation (FACT-BMT) was used due to its subscale for the evaluation of QoL in patients after HSCT. Final result of FACT-BMT is score ranged 0–148 point (the higher the score, the better QoL). For qualitative assessment of donor–recipient relationship, the adult sibling relationship questionnaire (ASQR) in Polish version was used. The ASQR-S consists of 47 items which are spread over eight scales designed to investigate three factors: warmth, conflict and rivalry. The questionnaires were given to both subgroups, donors and recipients of MSD-HSCT and the results were compared to each other. The overall result of the FACT-BMT questionnaire was  $109.0 \pm 7.5$  points, which means that the examined group generally described their QoL as ‘quite good’. The best results were found in functional well-being ( $25.6 \pm 0.9$ ), while the worst in emotional well-being ( $20.7 \pm 0.5$ ) dimension. Statistically, the QoL score was not influenced by age at HSCT ( $P=0.256$ ), current age ( $P=0.378$ ) or gender ( $P=0.117$ ) of the respondents. The recipients scored highest on warm factor ( $62.6 \pm 7.8$ ), while donor respondents scored slightly higher rivalry ( $60.3 \pm 6.0$ ) than warm ( $45.7 \pm 5.4$ ). The second dimension scored by recipients was rivalry ( $40.7 \pm 6.8$ ). Conflict scores were lowest, although donor respondents scored higher on these than recipient respondents ( $38.6 \pm 5.5$  in donors vs  $32.9 \pm 3.6$  in recipients). Statistical analysis revealed that the being a donor or recipient of MSD-HSCT determines the level of rivalry in the sibling relationship ( $P=0.007$ ) with no impact on warm and conflict dimension. Health-related QoL in transplanted patients is quite good. Sibling donor–recipient relationship is unbalanced with recipient respondents being more likely to assess a warm relationship, while rivalry was more likely to be present among donor. Further multicenter studies based on larger cohort of patients are necessary to assess sibling relationship after transplantation life experience.

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**P370****Rate of re-admission in patients undergoing allogeneic transplants from identical siblings, unrelated donors or haploidentical donors**

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HLA identical siblings (SIB), unrelated (UD) and family HLA haploidentical donors (HAPLO) are currently being used for patients undergoing an allogeneic transplant (HSCT) for hematologic disorders. GvHD prophylaxis is usually different, and is commonly based on a calcineurin inhibitor (CNI) and methotrexate (MTX) with or without ATG for SIBs and UDs, whereas post-transplant cyclophosphamide (PT-CY)+a CNI and mycophenolate (MMF) is used for HAPLOs. We will refer as SIB, UD, HAPLO platform, the combination of a given donor and a given GvHD prophylaxis. The outcome of these three different platforms is usually measured in terms of GvHD, non relapse mortality (NRM) and survival. Days of admission and re-admissions are important in terms of morbidity, but also of costs, and are usually not reported. Aim of the study: assess the duration of the first admission and the incidence of a new re-admissions, in the first 100 days after the transplant. We retrospectively analyzed 151 patients from 2012 to 2016. Sixty-one received peripheral blood stem cell graft from an UD, and GvHD prophylaxis with CyA+MTX+ATG; 54 received a peripheral stem cell graft from a SIB and GvHD prophylaxis with CyA+MTX; 36 patients received bone marrow HSCT from HAPLO-related donor and PT-CY+CyA+MTMF. Patients characteristics are shown in Table 1. Relapses were excluded from the re-admission analysis. The median time from the transplantation to discharge was 25 days for UD, 27 for HAPLO and 21 days for SIB: there was no significant difference between HAPLO vs UD ( $P=0.6$ ), whereas the admission of both HAPLO and UD was longer than SIBs ( $P < 0.01$ ). First readmission. Fiftyone patient out of 151 required of a new admission for complications after transplant (28 out of 61 after MUD (46%), 13 out of 54 (24%) using a sibling donor and 10 out of 36 using an haploidentical donor (28%). There were significantly more re-admissions in the UD vs SIB group (0.01) and a trend for more UD re-admissions vs HAPLO ( $P=0.08$ ); siblings had the lowest number of readmissions. Time to neutrophil engraftment was comparable in HAPLO vs UD patients ( $P=0.1$ ) and in SIB vs UD ( $P=0.1$ ); the time was longer in HAPLO vs SIBs ( $P < 0.01$ ). The reason to re-admitted the patients in the hospital after transplantation was fever in 14 out of 28 (50%) new admissions in UD setting, 11 out of 13 (85%) in SIB and 7 out of 10 (70%) in HAPLO; acute GvHD was the cause for re-admission in 5 out of 28 (18%) UD, 1 out of 13 (8%) SIB and none in HAPLO. The other causes for re-admission in the hospital were hemorrhagic cystitis, thoracic or abdominal pain. Second re-admission. of hospitalization is registered in 10 out of 61 patients in UD (7 for aGHvD and 3 fever), 2 out of 54 (4%) in SIB (2 episodes of fever) and 1 out of 36 (3%) patients in HAPLO (1 for fever and 1 progressive disease). Also for second episodes, UD grafts had significantly more admissions compared to HAPLO and SIBs. Third re-admission was recorded only in UD patients (5 out of 61–8%). This study shows a comparable duration of admission for transplant for HAPLO and UD patients, both significantly longer than SIB grafts. The number of re-admissions is comparable in HAPLO vs SIBs and there is a trend for lower number of re-admission as compared to UDs. We interpret this outcomes with caution given the relatively small sample size and heterogeneous disease population included. Future studies need to confirm our results.

**Disclosure of conflict of interest:** None.

**P371****Previously published****P372****Recombinant human thrombopoietin (rhTPO) for treatment of prolonged thrombocytopenia after allogeneic stem cell transplantation**

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Prolonged thrombocytopenia (PT) is frequent event after allogeneic haematopoietic stem cell transplantation (HSCT), especially in haploidentical transplantation, which could be up to 15% according to our previous report. PT has significant negative impact on long-term outcomes, mainly due to increased non-relapse mortality. However, there are no efficacious treatment. In this study, we report the preliminary results of recombinant human thrombopoietin (rhTPO) in treating this kind of patients. From 2016.7 to 2016.10, 16 patients were enrolled under the following inclusion criterion: (1) diagnosed with DPE or SFPR after allogeneic stem cell transplantation; (2) no sign of minimal residual disease or recurrence of hematological malignancy; (3) not using other TPO receptor agonist or IL-11 within 1 month of enrollment. PT include delayed platelet engraftment (DPE) and secondary failure of platelet recovery (SFPR). The former was defined as failure to achieve platelet counts  $\geq 20\ 000/\mu\text{L}$  for 7 consecutive without transfusion until 35 days after transplantation, while the latter was defined as a decline in platelet counts below  $20\ 000/\mu\text{L}$  for 7 consecutive days, or requiring transfusion support after achieving sustained counts without transfusions for 7 consecutive days after HSCT. The prescription of rhTPO was 15 000 IU once daily for 28 days, or if patients achieve platelet  $\geq 50\ 000/\mu\text{L}$  for 3 consecutive days with a duration  $< 28$  days. Response was defined as success of achieve platelet counts  $\geq 20\ 000/\mu\text{L}$  for 7 consecutive days. The response time was defined as the first day achieve response from the start of prescription. The primary end point was response rate, and the secondary end point was reponse time. A total of 16 patients were enrolled, including 7 males and 9 females. The median age was 30 (18–50) years. All patients received haploidentical transplantation. Among these patients, 10 patients were DPE and 6 were SFPR. All patients received a 28-day prescription. The overall response rate was 50% (8 out of 16) in the overall population, while 60% (6 out of 10) in DPE and 33.3% (2 out of 6) in SFPR, respectively. Among the 10 patients with response, the median response time was 21 (10–28) days from the first dose of rhTPO. After 4 weeks of the last dose of rhTPO, none of the responded patient lose response. Since the followup time is too short, the impact of relapse, GVHD were not reported. This single-arm preliminary result suggest that rhTPO could be a efficacious method to manage PT after stem cell transplantation. However, these result need further confirmation.

**Disclosure of conflict of interest:** None.

**P373****Reproductive health in long-term female survivors after allogeneic hematopoietic stem cell transplantation**

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Most female recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT) suffer from premature menopause, infertility and endocrine imbalance owing to gonadal damage from myeloablative conditioning. In order to evaluate ovarian recovery and long-term endocrine complications in our institution, we performed a retrospective study of female patients who received a myeloablative allo-HSCT during their reproductive age. We identified 50 female patients who underwent myeloablative allo-HSCT in our institution between 1983 and 2009 and were still alive with available follow-up at the time of this study. Among them, 37 patients accepted to participate and responded to a query designed for this

purpose. The median age of our patients at transplantation was 32 years (range: 12–47 years). They were interviewed at a median of 20 years (range: 7–33 years) post allo-HSCT. The majority of patients were transplanted for a myeloid malignancy (14 acute myeloid leukemia, 7 chronic myeloid leukemia, 3 myelodysplastic syndromes and 1 chronic myelofibrosis), while 7 patients had aplastic anemia and 5 had acute lymphoblastic leukemia. All patients received bone marrow transplant from a HLA-matched related donor after a myeloablative conditioning. Conditioning regimen consisted of cyclophosphamide with or without total body irradiation (TBI) or in combination with busulfan. Only 6 patients (16%) resumed a normal menstrual cycle after allo-HSCT, without the need for hormonal replacement therapy (HRT). All these patients were transplanted for aplastic anemia and none of them received TBI in the conditioning regimen. Eight patients (22%) remained amenorrheic indefinitely and never started HRT, even though most of these women were transplanted under the age of 40 years. 25% of these patients were diagnosed with osteoporosis later in life. The remaining 23 patients (62%) started HRT at a median of 11 months after allo-HSCT (range: 3–27 months). However, only seven patients on HRT (30%) resumed regular menstrual cycle. A median duration of HRT therapy was 6 years (range: 3–20 years). None of the women receiving long-term HRT had severe cardiovascular complications or breast cancer. Finally, five women gave birth to eight healthy children in our study population. Three unassisted pregnancies were observed in two female patients after spontaneous recovery of ovarian function (both patients with aplastic anemia). The remaining two patients restored ovarian function with the use of HRT and gave birth after an assisted pregnancy (one woman gave birth to triplets after an *in vitro* fertilization (IVF), while other became pregnant with a donated oocyte). In spite of the fact that almost all women who undergo allo-HSCT develop an ovarian failure, spontaneous recovery is sometimes possible, particularly following conditioning regimen without TBI. In patients without spontaneous recovery, HRT should be initiated promptly to prevent the early and late unwanted effects related to estrogen deficiency. Moreover, recovery of normal ovarian function and even a viable pregnancy is a realistic possibility in patients placed on HRT, particularly with the use of potential therapeutic interventions as IVF or oocyte cryopreservation. It is therefore crucial to provide adapted pre-transplant counselling and recommendations for regular post-transplant follow-up in female patients who undergo allo-HSCT.

**Disclosure of conflict of interest:** None.

#### P374

##### **Resolution of acute kidney injury secondary to TA-TMA by the anti-MASP-2 monoclonal antibody OMS721 in a pediatric HSCT recipient**

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Transplant-associated thrombotic microangiopathy (TA-TMA) is a multifactorial disorder caused by systemic vascular endothelial injury leading to end-organ damage often involving the kidney. TA-TMA occurs in up to 30% of patients undergoing HSCT, and may be associated with poor outcome. Although pathogenesis has not been fully clarified, activation of the complement system has been suggested to play a central role, and eculizumab, a monoclonal antibody (mAb) that mediates terminal complement blockade, has shown therapeutic benefit in cases unresponsive to

immunosuppression modulation. We report the case of a pediatric allogeneic HSCT recipient with severe TA-TMA, who did not tolerate treatment with eculizumab, now successfully treated with OMS721, a novel human mAb targeted to the mannin-binding lectin-associated serine protease-2 (MASP-2), a molecule central to the activation of the lectin pathway of complement. A 14-year-old girl received an allogeneic HSCT from a HLA-compatible unrelated donor for the treatment of Diamond-Blackfan anemia. At month +5 of the post-transplant course, she developed progressive deterioration of renal function, microhematuria and serositis, that prompted the cyclosporine discontinuation. From month +7, the patient experienced progressive trilinear cytopenia, elevated LDH, schistocytes, undetectable haptoglobin, hypertension, increased serum creatinine, nephrotic range proteinuria, and serositis, and a diagnosis of TA-TMA was established. Laboratory investigations documented no abnormalities in the patient but identified a stop-codon heterozygous 43 variant in CFHR5 c.485\_489dupAA (p.Glu163Lysfs\*10) in the donor's DNA. The patient was initially treated with eculizumab, but she developed acute pulmonary edema soon after eculizumab administration as the consequence of a possible reaction to the drug which had to be discontinued. The patient was subsequently treated with plasma exchange, with only limited benefit. Upon TA-TMA relapse at month +11, eculizumab was re-administered at lower doses, but she developed a new episode of acute pulmonary edema, preventing further eculizumab continuation. Renal function progressively deteriorated and she was started on hemodialysis, reaching a 3 times weekly regimen. The patient received OMS721, kindly provided on a compassionate use basis by Omeros Corporation, Seattle, USA, starting with an IV dosing schedule. She did not experience any adverse events, and was able to tolerate the treatment well. At 2 months from OMS721 initiation, she has shown improvement in LDH and haptoglobin levels, and, more importantly, her creatinine levels have normalized, allowing for complete discontinuation of hemodialysis and partial outpatient management. Anti-MASP-2 mAb OMS721 is a promising new option for the treatment of TA-TMA occurring after HSCT, and seems to have a safe profile also in the pediatric/adolescent setting.

**Disclosure of conflict of interest:** None.

#### P375

##### **Severe cytokine release syndrome after T-cell replete haploidentical transplantation with post-transplant cyclophosphamide is associated with increased death rate**

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Haploidentical stem cell transplant (Haplo-SCT) represents a potential curative strategy for several hematological malignancies. Haplo-SCT may represent an alternative option when a HLA matched-identical sibling (HLAid) or a matched unrelated donor (MUD) is not available. The syndrome of systemic inflammation, characterized by fevers, vascular leak, hypotension, and respiratory and renal insufficiency, in the context of elevated inflammatory markers and cytokine levels was previously described as cytokine-release syndrome (CRS)1. Recent publications have elicited the occurrence of CRS after haploidentical transplant, especially after peripheral blood stem cell graft, and its high-related mortality 2–4. Here we report the experience of our institution with CRS after Haplo-SCT. Between March 2014 and October 2016, we treated 29 patients with Haplo-SCT with a graft source represented by peripheral blood stem cells. We monitored the occurrence of CRS symptoms and utilize a previously described grading system 1, 4 starting from day 0, up to day 14 after transplant. Severe CRS is defined as grade 3 or higher because it requires

aggressive interventions and is characterized by oxygen requirement  $\geq 40\%$ ,  $> 3$  L nasal cannula, hypotension requiring high dose or multiple vasopressors, grade 3 renal toxicity or grade 4 transaminitis. Other characteristics comprise new-onset altered mental status without other explanation and new cardiomyopathy without wall motion abnormality. Results: 27 out of 29 patients experienced fever between day 0 and day 14 post transplant with most episodes (24 patients) occurring between day 0 and day 4. On day 7 after transplant, 3 patients had grade 3, 6 grade 2 and 19 grade 0 CRS, respectively. By day 14 post Haplo-SCT, 5 patients had CRS grade  $> 3$ , 5 grade 2 and 1 grade 1. Overall, the incidence of CRS any grade was 43% (95% CI 21–55%). 1 year after transplant 8 patients died because of non-relapse related side effects. With a median follow-up for alive subjects of 10 months, 1-year overall survival (OS) was 64% (95% CI: 42–80%). 1-year OS was 73% for patients with a CRS 3 on day 7 ( $P=0.007$ ). Conclusions: CRS represent an important complication after Haplo-SCT. CRS score  $> 3$  on day 7 after HST apparently correlates with long-term survival. Better strategies need to be implemented for an early detection of severe CRS in order to develop effective treatments, such as tocilizumab, for this important side effect. Further studies are ongoing at our institution in order to correlate post-Haplo CRS with graft composition, laboratory parameters and immune-reconstitution.

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#### P376

##### Sexual functioning during the first year post-transplant: comparison between allogeneic and autologous survivors

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Hematopoietic cell transplantation (HCT) is associated with significant morbidity that impairs survivor's sexual functioning. However, few studies have specifically addressed it. Thus, we examined (1) sexual functioning during the first year post HCT, (2) differences between allogeneic and autologous HCT, and (3) whether demographic, clinical and psychological variables were associated with sexual functioning. This is a prospective multicenter study assessing patients before HCT, at day 90, 180 and 360. Sexual functioning was assessed with the Changes in Sexual Functioning Questionnaire, which yields a total score, along with scores for the dimensions of frequency, pleasure, orgasm, desire and arousal. Anxiety and depression (HADS) were also collected. We included 159 consecutive HCT recipients: 91 (53%) were men, with a median age of 51 years (range: 18–71), 93 (58%) received an allogeneic HCT and 66 (42%) an autologous HCT. Sexual functioning was significantly affected: 86% of the sample reported impairment at pre-HCT, 91% at day 90, 87% at day 180 and 86% at day 360. Mixed model analysis indicated that sexual functioning was not associated with time from HCT ( $P=0.802$ ) or HCT type ( $P=0.538$ ). However, there was an interaction between these two variables ( $P=0.022$ ), particularly at day 90, since sexual functioning had improved among autologous survivors and worsened among allogeneic survivors leading to non-significant differences between HCT type ( $P=0.082$ ). Frequency of sexual functioning improved during the study

period ( $P < 0.001$ ), and no differences were observed between HCT type ( $P=0.111$ ). Again, there was a borderline interaction between post-HCT time and HCT type ( $P=0.059$ ), since autologous survivors reached higher frequencies than allogeneic survivors, with significant differences at day 90 ( $P=0.003$ ). Pleasure significantly improved during the study period ( $P=0.035$ ), without observing differences between HCT groups ( $P=0.121$ ). Again, however, autologous survivors reported significant improvements in pleasure at day 90 ( $P < 0.001$ ) and a trend at day 180 ( $P=0.093$ ) when compared with allogeneic survivors. Orgasm did not improve during the study period ( $P=0.837$ ), and no differences were obtained between HCT groups ( $P=0.413$ ). Allogeneic survivors had higher orgasm scores at pre-HCT ( $P=0.020$ ), which worsened during the study period, particularly at day 90 ( $P=0.028$ ). In contrast, autologous survivors reported improvements in orgasm by day 90. Non significant results were obtained in the sphere of sexual desire and arousal ( $P > 0.1$ ). Bivariate analyses indicated that women, older age and depression were associated with impaired sexual functioning at all assessed time-points ( $P < 0.05$ ). Chronic graft-versus-host disease (GvHD) was associated with worse sexual functioning at day 180 ( $P=0.045$ ) and 360 ( $P=0.020$ ). No differences were obtained when considering diagnosis, having received previous HCT, intensity of the conditioning regimen and whether patients lived with a partner ( $P > 0.05$ ). Stepwise multivariate regression analyses indicated that gender ( $P=0.001$ ) and extensive chronic GvHD ( $P=0.012$ ) predicted for worse sexual functioning at day 360. Sexual functioning should be routinely assessed and considered for eventual targeted intervention in both HCT populations, particularly during the first year post transplant. Additional clinical efforts should focus on patients more vulnerable to impaired sexual functioning.

**Disclosure of conflict of interest:** None.

#### P377

##### Significant improvement of QoL by using ATG as part of the conditioning regimen followed by HLA-identical peripheral stem cell transplantation in acute leukemia patients. Results from a prospective, randomized phase III study (ATG family study)

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cGVHD is a major complication after allogeneic SCT. We previously demonstrated that the addition of anti-T-lymphocyte globulin (ATLG Neovii, formerly ATG-Fresenius) to a myeloablative preparing regimen followed by peripheral-blood SCT from an HLA-identical sibling for pts with acute leukemia resulted in a significant reduction of cGVHD, without increasing the risk of relapse or infection.<sup>1</sup> The study protocol included quality of life (QoL) questionnaires (EORTC QLQ-30 and HDC29) before and after SCT (day+ 100, 6, 12 and 24 mos). The QLQ-C30 includes a global QoL scale, five functional scales (physical, role, emotional, cognitive and social function) and nine symptom scales (fatigue, nausea-vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea and financial problems). The QLQ-HDC29 includes six multi-item scales and eight single items that describe impairment through high-dose treatment. Mixed models for repeated measures (MMRM) and linear mixed models (LMM) were used to analyze the time courses and the slopes of the outcomes depending on treatment arm (ATG vs non ATG), age, country, sex, and cGVHD. (ClinicalTrials.gov: NCT00678275). Pts with a QoL form returned decreased by visit (70% pre-SCT, 45% at 100 days and 29% at 24 mos after SCT). Forty-nine percent in the ATG and 60% in non ATG arm provided any QoL forms after SCT. Return of any post-SCT QoL forms by country was 68% for Germany, 62% for Italy and 25% for Spain. Pts with cGVHD were more likely to return QoL questionnaires (66% vs 45% w/out cGVHD) while neither age nor sex were closely associated with QoL form return. The majority of subscales of the QLQ-30 indicated an average improvement of QoL and reduction of symptoms over time, notably in the ATG group. In an MMRM model controlling for country, age, sex and cGVHD, pts treated with ATG showed significantly more pronounced improvement of global health status/QoL over time compared to non-ATLG ( $P=0.02$ ), with a treatment group difference of  $2.8 \pm 3.9$  points (marginal mean  $\pm$  SEM) at day 100 and increasing to  $10.5 \pm 5.3$  points at month 24 favoring ATG. Significant superiority of ATG ( $P < 0.05$ ) was also observed for four of the five functional scales as well as for several symptom scales including appetite loss, insomnia, nausea-vomiting and dyspnea. For the QLQ-HDC29, significant treatment effects favoring ATG were observed for GI side effects and impact on family. LMM analyses of QoL by country indicate that patients from Italy generally gave more favorable ratings for all functional scales and lower scores for most symptom scales than those from Germany while the time courses and slopes were similar for most scales. These results underline the importance of the habits and cultural environment which are distinctive of each country. Males and females showed similar QoL ratings at pre- and post-SCT. Patients up to 34 years tended to provide more favorable functional ratings and less severe symptom scores than older patients and also showed more pronounced improvements of QoL. Pts receiving ATG in a randomized study have significantly less cGVHD and improved GFRS, resulting in an improved QoL regarding global health status and most functional scales. Notably, we also observed a significant difference in QoL assessment between pts from Germany and Italy.

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#### P378

##### The impact of oral mucositis on the quality of life of stem cell transplanted patients

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Oral mucositis (OM) is a well-known side effect of high-dose chemotherapy and radiotherapy in hematological patients, which influences the health-related quality of life (HRQoL) of the affected patients. The purpose of this study is to

demonstrate the impact of OM on HRQoL in stem cell transplanted patients in routine care. Prospective, non-interventional single-center observational study was performed at a German university hospital. Inpatient allogeneic and autologous stem cell transplant patients  $\geq 18$  years with high-dose chemotherapy. OM was assessed with the WHO Oral Toxicity Scale, pain using the Numeric Rating Scale (NRS) and the performance status with the ECOG Score. HRQoL was captured with the EORTC QLQ-C30 and the QLQ-OH15 questionnaires (3 days before hematopoietic stem cell transplantation (HSCT); 7 days after HSCT; 14 days after HSCT). Statistical significance was assumed  $P < 0.05$ . A total of 20 patients (11 autologous and 9 allogeneic) was included from August to December 2016. A total of 11 (55%) patients developed OM. Of these 11 patients, 3 suffered from Grade 1, 3 from Grade 2, 4 from Grade 3 and 1 from Grade 4 OM. Three days before HSCT, the mean QoL of all 20 patients was 45%, the mean QLQ-C30 Summary Score 60.3% and the mean oral health related quality of life 82.3%. Most of the patients suffered from OM around day 7 after HSCT. After 7 days, quality of life (QoL) was higher in patients with no OM (32.3%) than in patients with OM (30.0%). The QLQ-C30 Summary Score was significantly ( $P=0.004$ ) lower in patients affected by OM (43.1%) than in patients who did not develop an OM (65.8%). OM affected patients had significantly more limitations in emotional (no OM 84.4%; OM 56.7%;  $P=0.038$ ) and cognitive functioning (no OM 93.8%; OM 51.7%;  $P=0.002$ ) and in fatigue (no OM 58.3%; OM 80%;  $P=0.045$ ), pain (no OM 14.6%; OM 55%;  $P=0.005$ ) and insomnia (no OM 12.5%; OM 60%;  $P=0.001$ ), they had a significantly higher rate of problems. Oral health-related quality of life was significantly ( $P=0.003$ ) lower in patients who were affected by OM (57.9%) compared to patients who did not develop an OM (83.9%) and patients with an OM had significantly more problems with a sore mouth (no OM 8.3%; OM 46.7%;  $P=0.028$ ), sticky saliva (no OM 29.2%; OM 63.3%;  $P=0.045$ ) and sensitive mouth (no OM 8.3%; OM 56.7%;  $P=0.003$ ). After 14 days, QoL was higher in patients with no OM (47.9%) compared to patients with OM (46.7%). Patients with no development of OM had a higher but not significant physical functioning, cognitive functioning and social functioning. Patients affected by OM had higher levels of fatigue and pain and more often suffered from a sore mouth. Oral health-related quality of life was higher in patients without OM (75%) compared to patients with OM (68.3%). Comparing all assessed days patients with OM had higher scores on the NRS increasing with a higher grade of OM (mean NRS score grade 1; 2–2.3, grade 3; 4–4.5), the ECOG Index was higher in OM affected patients during episodes with OM (mean ECOG score—2.3) compared to episodes without OM (mean ECOG score—1.9). OM has a major impact on the HRQoL, health-related symptoms and functionality. In the future, there has to be a higher awareness from clinicians and patients of the prevention, assessment and causes of OM. More research has to be initiated to ease the symptomatology and to improve patients' quality of life.

**Disclosure of conflict of interest:** None.

#### P379

##### Software-based image analysis of CT scans in patients after allogeneic stem cell transplantation for pulmonary chronic 'graft-versus host' disease diagnosis

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According to EBMT data, chronic GVHD (cGVHD) occurs in 40–70% of all patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Pulmonary cGVHD is the most severe form. But it is very unpredictable to use due to the fact

that many factors can affect it (breath-dependent; need experience not only from physician but from patients also and so on). Here we show that routine software-based image analysis algorithm can provide data that highly correlated with PFT results and have excellent sensitivity and specificity in pulmonary cGVHD diagnosis. We blindly analyzed 120 CT scans (made without additional expiration) in 24 allo-HSCT patients at different time points. All scans were performed on CT Scanner Aquilion 64, Toshiba, Japan. According to Hounsfield units (HU) definition, -1000 HU ('air') have approximate density at 0 g/mL; 0 HU ('water') have approximate density at 1 g/mL. The analysis of CT scans (heart, vessels and bronchi were excluded from analysis) was based on automated software conversion (image-analysis algorithm providing by Multivox Software, MSU, Moscow, Russia) of each CT-image pixel from HU to density units (g/mL). PFT were performed using standard procedures at same as CT scans time points (Spirolab III, Italy). All patients with hematological malignancies (acute leukemia—17, aplastic anemia—1, chronic myeloid leukemia—1, T-cell lymphoma—1, chronic myeloproliferative disorder—1, myelodysplastic syndrome—3) were transplanted in National Research Center for Hematology between 2012 and 2015. Median of age was 41.5 years (range: 19–60 years). Eight patients were males, 16—females. Seventeen received reduced-intensity and 7—myeloablative conditioning regimen. Graft from match unrelated donor (MUD) were used in 17 cases, 'mismatch' MUD—2, match related donor (MRD)—9, 'mismatch' MRD—1. Median follow-up is 44.8 months. We analyzed lung tissue experimental density in patients before and after allo-HSCT at different time points. Median of lung tissue experimental density were 0.178 (interquartile range (IQR), 0.148–0.186), 0.17 (IQR, 0.153–0.185) and 0.147 (IQR, 0.131–0.172) for patients before allo-HSCT, after allo-HSCT with cGVHD (except pulmonary cGVHD) and with pulmonary cGVHD, respectively. Mann-Whitney *U* test was used to reveal significant differences between these groups (see Figure 1). Also, we found strong correlation between PFT and experimental density (Spearman's correlation coefficient  $r=0.537$ ) (see Figure 2). Forty-five CT scans of patients with pulmonary cGVHD and 59 CT scans of patients without pulmonary cGVHD at the time of CT scan as control subjects were included in ROC analysis to assess the clinical values of our model. We generated an ROC curve and found that the area under the curve (AUC) was 0.77 (95% CI, 0.67–0.86) ( $P < 0.0001$ ) (Figure 3). Standard CT scan is presented as easy to perform, breath-independent, standardized and wide spread method for every patient after allo-HSCT. It can be performed many times during all their post-HSCT life. CT scan with a simple software analysis allows to select a group with high probability of pulmonary cGVHD and who can be suspected of cGVHD development by this method with sensitivity—60% and specificity—81.36%.

**Disclosure of conflict of interest:** None.

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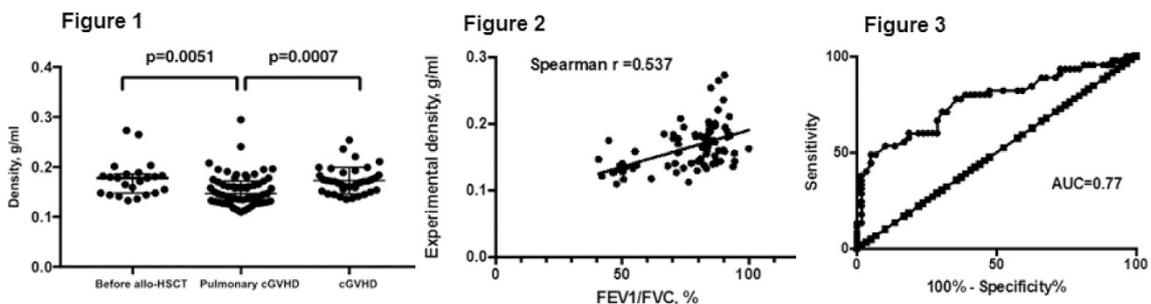
#### The choice of effectiveness criteria affects conclusions of economic evaluation of newer allogeneic bone marrow transplantation modalities :example based on a randomised multicenter trial comparing two reduced intensity conditioning regimen (FLU-BU-ATG) vs (FLU-TBI) for matched related allo-SCT

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In medico-economic evaluations, the recommended effectiveness criterion is the quality adjusted life year (QALY). To be constructed, the QALY needs the overall survival (OS) and quality of life weighting coefficients. OS takes years to observe, therefore intermediary end points like disease- or progression-free survival (PFS) are often used and are of growing use in the economic evaluation of advanced cancer literature. Our study compared cost-effectiveness analyses using three different effectiveness criteria : the PFS, the OS and the QALY on the basis of a multicenter randomized trial comparing two reduced intensity conditioning regimen for matched related allo-SCT published in 2013 (Blaise *et al. Cancer* 2013; **119**: 602–611). The ITAC01 study prospectively compared 2 RIC (FBA (Fluda+Oral Bu+Thymoglobuline) or FTBI (Fluda+2 Gy TBI) with 1 year OS as primary end point. A total of 139 patients were treated (FBA:  $N=69$ ; FTBI:  $N=70$ ). Groups were comparable. Direct medical transplant costs were estimated by micro-costing on the basis of patients' CRF until 18 months after transplant from the hospital point of view. Costs of treatment of progression were estimated within 5 years after transplant. A re-evaluation of the per-diem hospitalization cost was performed in 2016 and included the utilization of hospital technical facilities and a more precise estimation of overheads costs. We performed three separated cost-effectiveness analysis, using, respectively, PFS, OS and QALY as end point. When using PFS as effectiveness, relapse costs were not included. Weighting coefficients for the cost per QALY analysis came from the literature. At 5 years, OS and PFS were 41% and 29%, respectively, and did not statistically differ between groups. The mean total cost per patient was not statistically different between groups (111725€ for FBA vs 98316€ for FTBI, NS). Using PFS as end point, the ICER of FBA compared to FTBI is 35 034€ per year of PFS gained. Using OS, the ICER became non-statistically significant, signifying that when handling uncertainty, no difference in term of cost-effectiveness was observed between FBA and FTBI with OS as end point. Using

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QALY, the ICER was statistically NS again, showing no advantage in terms of cost per QALY of one conditioning regimen over the other. This result was obtained both considering three weighted health states (DFS, progression and death) and four weighted health states (DFS without GVHD, DFS with GVHD, progression and death) for the QALY calculation. Using OS and QALY, the two conditioning regimens were not different in terms of cost-effectiveness, while FBA may be considered as more cost-effective using PFS as effectiveness criterion. Using intermediary end points allows economic evaluation to be available earlier in the life cycle of an innovation. However, it implies strong hypotheses about the predictive value of the PFS over the OS. Longer period evaluation and QALY may reverse preliminary results. This situation is likely to exist in the hematology setting where alternatives between chances of cure and toxicities of treatment are often observed. Research about allogeneic SCT modalities is archetypical of such situations and decisions makers should be aware of the necessity of further economic re-evaluation along the development and diffusion process of innovative treatments.

**Disclosure of conflict of interest:** None.

### P381

#### The impact of corticosteroids prophylaxis for the engraftment syndrome incidence during autologous stem cell transplantation in multiple myeloma and amyloidosis

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The ES is a complication of ASCT characterized by an inflammatory response during peripheral blood recovery. The standard treatment is based on corticosteroid therapy. The incidence of ES after ASCT increases in chemotherapy low-treated patients such as those with multiple myeloma (MM) and amyloidosis (AL). Moreover, the ES is associated with the use of G-CSF after infusion of stem cells. Therefore, our BMT team does not use G-CSF since 2009 in this population reducing the incidence and severity of ES. Therefore, it makes sense to use low-dose prednisone to prevent this complication. In this study, we compared two consecutive cohorts of patients with MM/AL that performed an ASCT while evaluating the corticosteroids prophylaxis (CP) in the ES incidence and its effect on other clinical variables. We included 120 patients with MM ( $n=96$ ; 80%) and AL ( $n=24$ ; 20%) that performed an ASCT between January 2011 and November 2016 in a single institution. The median age (range) was 56.7 (33.8–71.7) years. During the procedure, all patients received melphalan as conditioning chemotherapy and none received G-CSF. Forty-seven patients (39%) received intravenous methylprednisolone or oral prednisone 0.5 mg/kg/day from day +7 until reaching a neutrophil count  $\geq 500$  per  $\text{mm}^3$  for 3 consecutive days (CS group), and 73 (61%) patients did not receive corticosteroids (NonCS group). The characteristics of patients in both groups (age, gender, status performance and previous treatment) were similar ( $P > 0.05$ ). The CS group, received higher doses of  $\text{CD34}^+$  than the NonCS group ( $3.68 \times 10^6/\text{kg}$  vs  $2.92 \times 10^6/\text{kg}$ , respectively,  $P=0.006$ ). The median (range) days of neutropenia ( $< 500$  per  $\text{mm}^3$ ) was 9 (4–25) days. ES was diagnosed in 43 (36%) patients. Fifty-seven (48%) patients had fever, showing infectious focus or microbiological isolation in 24 (20%) cases, whereas the incidence of grade III–IV oral mucositis and relevant gastrointestinal toxicity was 8% and 2.2%, respectively. The complete analysis between groups (CS versus NonCS) for the whole series and in the MM/AL subgroups is detailed in Table 1. The administration of corticosteroids as prophylaxis seems to reduce the incidence of ES in the overall series or in the analysis for the subgroups (MM and AL) without increasing infection.

[P381]

Results (global series)				
	NonCS, N=73 N (%)		CS, N=47 N (%)	
ES*	35 (48) *		8 (17) *	
Neutropenia $< 500/\text{mm}^3$ , median (range)	9 (5-25)		9 (4-16)	
Fever	37 (51)		20 (43)	
Infection	17 (23)		7 (15)	
Mucositis GIII-IV	5 (7)		1 (2)	
Gastrointestinal toxicity GII-IV	2 (3)		1 (2)	
$\text{CD34}^+ \times 10^6/\text{Kg}^*$ Median (range)	2.92 (1.92-9.63)*		3.68 (1.94-8)*	
Results according to MM vs. AL				
	MM, N=96		AL, N=24	
	NonCS, N=61 N (%)	CS, N=35 N (%)	NonCS, N=12 N (%)	CS, N=12 N (%)
EF*	25 (41) *	7 (20) *	10 (83) *	1 (8) *
Neutropenia $< 500/\text{mm}^3$ , median (range)	9 (5-25) *	8 (4-16) *	7 (6-13)	10 (6-13)
Fever	31 (51)	2 (20)	6 (50)	6 (50)
Infection	14 (23)	1 (10)	3 (25)	2 (16)
Mucositis GII-IV	4 (7)	0 (0)	2 (20)	1 (12)
Gastrointestinal toxicity GII-IV	1 (2)	0 (0)	0 (0)	1 (12)
$\text{CD34}^+ \times 10^6/\text{Kg}$ Median (range)	3 (1.92-9.63)	3.41 (1.94-8)	2.58 * (2-8.85)	5.22* (2.8-7.75)
* $P < 0.05$				

**Disclosure of conflict of interest:** None.

### P382

#### The incidence and outcome of post-transplant lymphoproliferative disorders do not limit the use of low/intermediate-dose ATG if systematic and close monitoring of EBV viremia is performed

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Chronic GVHD is a condition that might occur after allo-HSCT and has been proved to impair long-term survival and quality of life of patients. Graft failure is also a major potential complication for patients undergoing transplant for an aplastic anemia/bone marrow failure (BMF). Partial *in vivo* T-cell depletion, employing anti-thymocyte globulin (ATG) during conditioning, has been proved to successfully prevent the mentioned potentially life-threatening complications in high-risk patients. However, the possibility of developing Epstein-Barr virus (EBV)-induced post-transplant lymphoproliferative disorders (PTLP) has been a limiting factor to use ATG. This study includes the last 100 pts with a minimum follow-up of 100 days, who underwent allo-HSCT in our center (November 2014–August 2016). A total of 56 pts were male and 44 female. Median age was 53 years (range: 7–69). Baseline diseases were: acute leukemias (54), lymphoproliferative disorders (17), myelodysplastic syndromes (12), chronic myeloproliferative diseases (7), multiple myeloma (5) and bone marrow failures (5). Donor was unrelated in 57 cases, and related in 43 (including 18 haplo-identical). Conditioning regimen was: busulphan-based (70), melphalan-based (13), TBI-based (8) and others (9). Progenitors source was PB in 89 and BM in 11. Patient/donor EBV pre-transplant serology was: ++ in 94 cases, +/- in 5 and -/+ in 1. Rabbit ATG (thymoglobuline) was employed in 58 cases: 54 at 4.5–6 mg/kg (URD transplants) (low dose), and 4 cases at 7.5 mg/kg (all of them pts with BMF) (intermediate dose). Family donor (including

haplo-identical) transplants of those pts with diagnosis different from BMF (42 cases) did not receive ATG. Systematic monitoring of EBV using quantitative PCR was employed. EBV reactivation was considered when DNAemia was superior to 1000 copies per mL. A total of 5 pts presented EBV reactivation: 0/42 (0%) in cases without ATG, 4/54 (7.4%) in cases with low-dose ATG and 1/4 (25%) in cases with intermediate-dose ATG. Median time of reactivation was the day +34 (range: +32 to +151). There was one single case of EBV-induced PTLD which belonged to the intermediate-dose ATG group. All cases (including the one with PTLP) were successfully treated with Rituximab at 375 mg/m<sup>2</sup>/week. Median number of doses employed were 3 (range: 2–19). Mortality due to EBV was 0% in our series. (1) Reactivation of EBV was clearly linked to the use and dose of ATG; (2) close monitoring of the EBV viremia and pre-emptive treatment with Rituximab in case of reactivation results in a very low rate of EBV disease and, in our experience, no mortality in patients receiving low-intermediate dose of rATG; (3) in those conditions, EBV disease would not limit the use of low-intermediate dose of rATG to prevent incidence and severity of cGVHD in URD transplant patients and to promote the graft in BMF patients.

**Disclosure of conflict of interest:** None.

### P383

#### The outcome of haploidentical hematopoietic cell transplantations

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Limited donor availability in the form of either matched-related or unrelated donors drew attention to haplo-HCT. Donors of haplo-HCT shares an exact haplotype with the recipient but is mismatched for HLA genes on the unshared haplotype. Most studies have shown promising results in terms of graft success and survival. In this study our aim is to present the early and late outcome of our haplo-HCT patients. Between 2012 and 2016, we retrospectively evaluated 16 haplo-HCT in terms of post-transplant outcome, survival and complications who diagnosed and followed in our center. The median age of patients was 37 (range: 19–61), 10 (63%) of them were male recipients. The patient characteristics were given in Table 1. Thirteen patients (81%) had pre-transplant active disease. Neutrophil and platelet engraftment was achieved in 7 patients (44%) at a median day of 21 (range: 16–40) and 34 (range: 13–116). Eight of 16 patients (50%) died within 1 month after transplant because of sepsis without achieving engraftment. Haplo-HCT is the second transplant in four of 16 patients (25%): 1 patient relapsed after full-matched related transplant, 1 patient relapsed after 9/10 matched unrelated transplant, 1 patient had engraftment failure after full-matched unrelated transplant, 1 patient underwent haplo-HCT in another center, followed in remission for 2 years and relapsed. Acute graft vs host disease (aGVHD) was diagnosed in 6 patients (37%), whereas chronic GVHD in 4 patients (25%). Four patients were relapsed (25%) during follow-up with median RFS of 6 months. Three patient had BK virus-positive hemorrhagic cystitis (18%). The distribution of infections is shown in figure, viral infections were detected later than fungal and bacterial infections. Previous history of invasive pulmoner aspergillosis was detected in 5 of the patients (31%) (2 of them were re-transplanted) and received secondary prophylaxis. Overall survival (OS) of 6 months and 1 year were 25% and 18%, respectively. The choice between alternative graft sources depends on the urgency of the transplant on each institutional preference. Higher complication and

infection rates in addition to decreased survival compared with previous studies since our patient population consisted of refractory patients with comorbidities. Preferable patient profiles undergoing haplo-HCT may have better outcomes.

**Disclosure of conflict of interest:** None.

### P384

#### The third month risk factor score: detection of disease at day +100 of allogeneic stem cell transplantation is the most important risk factor of worse prognosis

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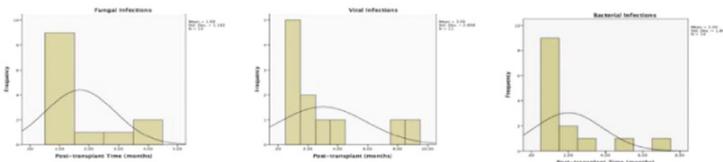
Before allogeneic stem cell transplant (SCT), several index can provide prognostic information (EBMT risk score and HCTI score). However, there is scarce data for the impact of the procedure during the first 100 days of transplant, in which opportunistic infections and the acute graft versus host disease (GVHD) can induce harmful effects. Our purpose is to create a risk factor score, measured at day +100 post SCT, to give information about the prognosis of the patient. We retrospectively analyzed seven clinical (disease, fungal and CMV infection, acute GVHD, treatment with corticosteroids, Karnofsky status and length of hospitalization) and eight analytical (related to immune status, liver and lung function, nutritional status, iron overload and platelet count) risk factors in 131 patients who underwent SCT in our center between 2011 and 2015 and were alive at day +100. Data were collected as categorical variables and compared by  $\chi^2$ -test. Significant variables ( $P < 0.05$ ) were evaluated in a multivariate logistic regression model. Those who maintained statistical significance were then assigned a point value calculated with their  $\beta$ -coefficient. Summation of the points resulted in a weighted risk score. Median age was 51 years (range: 4–73) and 80 were males (61.1%). The most frequent disease was AML, 49 patients (37.4%). The conditioning regimen was myeloablative in 97 patients (74%) and bone marrow was the principal stem cell source (71%). Donor was MRD in 39 (29.8%), MUD in 51 (38.9%) and MMD in 41 (31.3%). The median follow-up was 26 months (range: 3–66). The univariate model identified five prognostic variables: detection of disease by molecular, cytogenetic or flow cytometry asses in leukemias, myelodysplastic syndrome and multiple myeloma or image (CT scan  $\pm$  PET) in lymphoma, dose of corticosteroids  $\geq 0.5$  mg/kg/day, ferritin  $> 2500$  ng/mL, albumin  $< 3.0$  g/dL and platelet  $< 100\,000$  per mm<sup>3</sup>. Table 1 shows variables evaluated. In the multivariate model, the detection of disease (HR 3.80, 95% CI 1.90–7.61,  $p < 0.005$ ) and platelet  $< 100\,000$  per mm<sup>3</sup> (HR 2.06, 95% CI 1.11–3.89,  $P = 0.022$ ) were associated with higher risk of death and according with their—coefficient 4, 2 and 2 points were, respectively, assigned. The third month risk score (TMRS) was calculated in all patients and they were stratified into three groups: low risk of death (A, 0–2 points), intermediate risk (B, 4 points) and high risk (C,  $\geq 6$  points). At 2 years post SCT, the estimated overall survival according with the TMRS was 78.9%  $\pm$  4.3 in group A, 31.0%  $\pm$  8.6 in group B and 36.4%  $\pm$  14.5 in group C,  $P < 0.001$ . Although the harmful effect of the first 3 months of transplant can impact in the survival, the detection of disease at day +100 is the most determinant risk factor of death. This fact gives us the need of transplant in the best response and, in those who cannot, to plan promptly rescue strategies. The next objective is to confirm our risk score in a validation group.

**Disclosure of conflict of interest:** None.

**Table.** Patient Characteristics (ATG:Antitimosit Globulin, Bu:Busulphan, CSA: Cyclosporin, Cy: Cyclophosphamide, Flu:Fludarabine, FLAMSA:Fludarabine,Amsacrine,Cytarabine Mel:Melphalan, MMF: Mycophenolate Mofetil, TBI:Total Body Irridation)

Characteristics	Frequency (n,%)	Posttransplant Complications	Frequency (n,%)
<b>Diagnosis</b>		<b>Acute GVHD</b>	
Acute Leukemia	13 (82%)	Grade III Gastrointestinal	1 (6%)
Myelodysplastic Syndrome	1 (6%)	Grade II Skin	4 (25%)
Chronic Myeloid Leukemia	1 (6%)	Grade III Skin	1 (6%)
Aplastic Anemia	1 (6%)		
<b>Stem Cell Source</b>		<b>Chronic GVHD</b>	
Peripheral Blood	12 (75%)	Gastrointestinal	4 (25%)
Bone Marrow+Peripheral Blood	3 (19%)	Skin	2 (12%)
Bone Marrow	1 (6%)		
<b>Donor Type</b>		<b>Viral Infections</b>	
Parents	8 (50%)	Sitomegalovirus (CMV)	7 (44%)
Children	6 (38%)	BK virus	3 (19%)
Sibling	2 (12%)	Others (Adenovirus, Rhinovirus)	4 (25%)
<b>Conditioning Regimen</b>		<b>Fungal Infections</b>	
Flu+Mel+Thiotepa	7 (44%)	Aspergillus spp	4 (25%)
Flu+Cy+TBI	2 (12%)	Candida spp	2 (12%)
Flu+Bu+TBI	2 (12%)	Mucormycosis spp	4 (25%)
Cy+TBI	1 (6%)	Zygomycetes spp	1 (6%)
Flu+ATG+Thiotepa	1 (6%)		
Flu+Cy+Bu	1 (6%)		
Flu+Mel	1 (6%)		
Flu+ATG+TBI	1 (6%)		
<b>Graft vs Host Disease Prophylaxis</b>		<b>Hemorrhagic Cystitis</b>	
Cy+Tacrolimus+MMF	10 (63%)		3 (19%)
Tacrolimus+MMF	3 (19%)		
CSA+Mtx	2 (12%)		
Cy+CSA+MMF	1 (6%)		
<b>Sorror Score (HCT-CI)</b>		<b>Thrombotic Thrombocytopenic Purpura</b>	
0	6 (38%)		
1	6 (38%)		2 (12%)
2	2 (12%)		
3	2 (12%)		

**Figure.** Infection distribution throughout months



**P385**  
**Therapeutic potential of recombinant human soluble thrombomoduline alpha for SOS/VOD: a retrospective analysis in Toranomon Hospital, Japan**

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Recombinant human soluble thrombomoduline alpha (rhTM) is a novel anticoagulant agent and approved for disseminated

intravascular coagulation in Japan. The aim of the study is to evaluate the therapeutic potential of rhTM for sinusoidal obstructive syndrome/hepatic veno-occlusive disease (SOS/VOD). We retrospectively studied 878 times of allogeneic hematopoietic cell transplantation in Toranomon Hospital from June 2008 to June 2015. We extracted the patients who used rhTM for DIC and satisfied the diagnostic criteria of SOS/VOD around the same time, because the use of rhTM for SOS/VOD alone is off-label. Data on the patients who used rhTM for > 3 days within 100 days after transplantation were analyzed. The patients who were already treated with rhTM before the emergence of the first symptom or sign of SOS/VOD, and who started rhTM over 30 days after the emergence of the first symptom or sign of SOS/VOD, were excluded from the

Table 1.

Risk factor	Dead (n=51)	Alive (n=80)	P value
Age > 40 years	41 (80.4%)	51 (63.8%)	0.051
Time > 12 months from diagnosis to SCT	26 (51.0%)	33 (41.3%)	0.275
Non sibling donor	35 (68.6%)	57 (71.3%)	0.792
Myeloablative conditioning	36 (70.6%)	61 (76.3%)	0.471
<b>Determinations at day +100</b>			
Disease detected	13 (25.5%)	6 (7.5%)	0.009
CD4 < 100/mm <sup>3</sup>	8 (21.6%)	21 (32.8%)	0.231
Hypogammaglobulinemia	34 (72.3%)	44 (55.7%)	0.063
Bil > 3 mg/dl or GOT or GPT > 5 ULN	5 (9.8%)	2 (2.5%)	0.070
DLCO or FEV1 < 65%	11 (22.0%)	14 (17.9%)	0.573
Previous acute GVHD III-IV	10 (19.6%)	6 (7.5%)	0.055
CC dose > 0.5 mg/Kg/day	17 (33.3%)	12 (15.0%)	0.018
Karnofsky < 80	7 (13.7%)	4 (5.0%)	0.079
Length of hospitalization > 40 days	8 (15.7%)	4 (5.0%)	0.060
Ferritin > 2500 ng/ml	20 (41.7%)	12 (15.4%)	0.001
Albumin < 3.0 g/dl	9 (17.6%)	2 (2.5%)	0.003
Platelet < 100.00/mm <sup>3</sup>	29 (56.9%)	26 (32.5%)	0.007
Probable or proven IFI	3 (5.9%)	2 (2.5%)	0.325
CMV reactivation	23 (45.1%)	31 (38.8%)	0.472

analysis. To diagnose classical SOS/VOD ( $\leq 21$  days after transplantation), we used two classical criteria of the modified Seattle and the Baltimore. For late-onset SOS/VOD ( $>$  day 22 of transplantation), we used the criteria of EBMT. We defined as severe SOS/VOD, if the patients had renal ( $\text{Cr} \geq 2$  times of baseline), respiratory ( $\text{SpO}_2 \leq 90\%$  or the need for positive pressure) or central nervous system failure until 2 weeks after the diagnosis of SOS/VOD. Complete response (CR) was defined as the resolution of all the symptoms and the signs in SOS/VOD diagnostic criteria. A total of 39 patients were extracted. The median age was 60 years (range: 27–72) and 27 patients (69%) was male. Donor cell sources were UCB ( $n=34$ ) and UBM ( $n=5$ ). Most of the prophylaxis regimen was the combination of ursodeoxycholic acid and dalteparin in 36 patients (92%). Classical SOS/VOD was diagnosed in 3 (8%) and 8 patients (21%) by the criteria of the modified Seattle and the Baltimore at the median day of 14 (range: 11–14) and 16 (range: 11–20), respectively. Twenty-eight patients (72%) were diagnosed as late-onset SOS/VOD at the median day of 44 (range: 22–89). Severe SOS/VOD developed in 33 patients (85%) (renal,  $n=32$ ; respiratory,  $n=7$ ; central nervous system,  $n=15$ ). The elevation of transaminase was observed in 18 patients (46%). The median interval from the emergence of the first symptom or signs of SOS/VOD to rhTM administration was 7 days (range: 0–23). The median duration of rhTM use was 11 days (range: 3–63). RhTM was used alone in 20 patients (51%), in combination with dalteparin in 7 (18%), with ATIII in 5 (13%), with dalteparin and ATIII in 3 (8%), with ATIII and PGE1 in 2 (5%), and with PGE1 in 2 (5%). Corticosteroid was used concomitantly in 32 patients (82%). Finally, 13 patients achieved CR of SOS/VOD. The cumulative incidence of CR of

SOS/VOD was 33.3 % at 1 year after the administration of rhTM (95% confidence interval, 18.5–48.9%). The median interval from the administration of rhTM to CR of SOS/VOD was 51 days (range: 6–141). At 1 year after transplantation, overall survival was 25.6% (95% confidence interval, 13.3–69.9%). From the administration of rhTM to 2 weeks after the cessation of rhTM, 23 hemorrhagic adverse events were observed. Seven out of 23 events were at grade 3–5, and 5 out of 7 events were fatal (intra-abdominal in 2, gastrointestinal in 1, lung in 1 and brain in 1). We concluded that rhTM had a therapeutic potential for SOS/VOD.

**Disclosure of conflict of interest:** None.

### P386

#### Thrombopoietin receptor agonists for delayed and prolonged clinically-relevant severe thrombocytopenia after allogeneic hematopoietic stem cell transplantation

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Persistent thrombocytopenia is a common complication after allogeneic stem cell transplantation (AlloSCT), which dramatically increases the patients' dependence on hospital-based healthcare. Thrombopoietin receptor agonists (TPOa) increase platelet counts in other clinical settings; however, the experience regarding their use after alloSCT is limited. We retrospectively evaluated TPOa efficacy in 15 consecutive adult

**Table 1.** Patient characteristics and outcomes

Number	Age	Gender	Disease	AlloSCT	Indication TPOa	TPO agent	Days from start TPOa to Platelets $\geq 30,000/\mu\text{l}$	Treatment duration (days)
1	69	F	MFI	PB/MUD	PFPR	Romiplostim	NR	23
2	49	M	CML	BM/MMUD	SFPR	Romiplostim	NR	56
3	16	F	DKC	BM/MUD	PFPR	Eltrombopag	NR	55
4	48	F	MM	PB/IS	SFPR	Romiplostim	NR	35
5	44	F	MDS	PB/IS	PFPR	Eltrombopag	NR	29
6	22	M	ALL	PB/HI	PFPR	Romiplostim	41	385
7	37	F	AML	PB/HI	SFPR	Romiplostim	15	14
8	38	F	AML	PB/HI	SFPR	Romiplostim	34	56
9	54	M	AML	UCB/MMUD	SFPR	Romiplostim	14	98
10	74	M	AML	PB/MUD	SFPR	Romiplostim	28	42
11	57	M	NHL	PB/MMUD	SFPR	Romiplostim	15	7
12	38	F	NHL	PB/MMUD	SFPR	Romiplostim	3	42
13	63	M	NHL	PB/IS	PFPR	Romiplostim	NR	24
14	30	F	HD	PB/MMUD	SFPR	Romiplostim	29	7
15	22	F	HD	PB/RMM	SFPR	Romiplostim	43	385

Abbreviations: F, female; M, male; MFI, myelofibrosis; CML, chronic myeloid leukemia; DKC, Dyskeratosis congenita; MM, multiple myeloma; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; PB, peripheral blood; BM, bone marrow; UCB, umbilical cord blood; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; IS, identical sibling; HI, Haploidentical; RMM, related mismatched; PFPR, primary failure platelet recovery; SFPR, secondary failure platelet recovery; NR, no-response.

alloSCT recipients who received TPOa as a compassionate use for severe thrombocytopenia post-engraftment. Five patients (33%) had primary and prolonged failure of platelet recovery, while 10 had secondary thrombocytopenia: in seven of these cases, GVHD and/or a viral infection were the 'attributed' cause, while three were classified as post-AlloSCT ITP. All 15 patients were dependent on platelet transfusions (median: 2 times per week, range 1–5), with severe bleeding episodes in nine cases (60%) before TPOa onset. TPOa was started at a median of 160 days after alloSCT (range: 65–1041). Romiplostim was used in 13 (87%) cases. The median starting dose was 2  $\mu\text{g}/\text{kg}$  once a week (range: 1–5  $\mu\text{g}/\text{kg}$ ), while the final dose identified as most beneficial was 4  $\mu\text{g}/\text{kg}$  (range 1–10  $\mu\text{g}/\text{kg}$ ). Eltrombopag was used in 2 cases (13%), with an initial dose of

50 mg daily; while the final doses were 100 and 150 mg daily. Overall, 9/15 patients responded to TPOa therapy (defined as a stable platelet recovery to  $\geq 30,000/\mu\text{l}$  without transfusion support). The 180-day cumulative incidence of successful platelet recovery to  $\geq 30,000/\mu\text{l}$  and  $\geq 50,000/\mu\text{l}$  was 70% (95% CI, 67–73%) and 56% (95% CI, 42–69%), respectively, which were reached at a median of 21 and 48 days from start of therapy. Five of the 9 patients (56%) with severe bleeding at onset responded to TPOa (4 of them without further hemorrhages) at a median of 93 days (range: 1–299). At a median follow-up of 511 days from start of therapy, three patients who responded continue TPOa treatment, while four other responders were able to discontinue it without recurrence of thrombocytopenia. Among these 4 patients,

the median total duration of treatment was 201 days (range: 113–300). One patient lost his response within 5 months after TPOa onset when he developed thrombotic microangiopathy associated with progressive GVHD. The remaining responder experienced disease relapse on day +88 after alloSCT. Among the 6 non-responders, 1 had leukemia relapse during TPOa treatment, 1 switched from romiplostim to eltrombopag without success and the remaining cases had active severe infections at TPOa onset (2 hemorrhagic cystitis and 1 CMV colitis) or non-controllable intestinal bleeding due to progressive GVHD. TPOa were well tolerated, with only 2 patients showing adverse events (grade 3 liver toxicity and grade 3 fatigue), which did not lead to any change in therapy. Six patients (40%) underwent follow-up bone marrow biopsies that did not display any increase in marrow fibrosis, including the 1 patient who had myelofibrosis prior to alloSCT. Although six patients in the study had active GVHD when TPOa was started, no patients showed worsening of GVHD. Our results support the safety and efficacy of TPOa for the treatment of persistent thrombocytopenia in alloSCT recipients. Further studies should compare the efficacy of romiplostim and eltrombopag and identify surrogate clinical and laboratory variables that are predictive of response to one (or both) of these TPOa.

**Disclosure of conflict of interest:** None.

### P387

#### **Thrombotic microangiopathy after allogeneic stem cell transplantation—comparison of currently used treatment concepts in adult patients**

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Hematopoietic stem cell transplantation (HSCT) associated thrombotic microangiopathy (TA-TMA) is a life-threatening complication of HSCT and occurs in 20–30% of patients. Although not fully understood, TA-TMA is caused by systemic activation of the alternative complement pathway. Until today, there is no standard therapy for TA-TMA. The most commonly used therapeutic approaches (classical treatment) are plasma exchange, rituximab or defibrotide. The anti-C5 monoclonal antibody eculizumab (EC) showed a high success rate in the treatment of aHUS and in pediatric transplant patients with TA-TMA. However, the use of EC in adult patients with TA-TMA is limited. Furthermore, no comparison between the classical treatment and EC has been published. Methods: we retrospectively analyzed 37 cases with TA-TMA treated in our center between 1999 and 2016. Inclusion criteria were diagnosis of TA-TMA according to the Choi-criteria significant organ damage and later on (2014–2016) evidence of systemic or local complement activation by measurement of serum sC5-9 levels or tissue biopsy. Patients diagnosed with TA-TMA between 1999 and 2011 ( $n=24$ ) received classical therapy, the second group (2014–2016) ( $n=13$ ) received EC guided by monitoring CH50 activity and serum sC5-9 concentrations as proposed by Jodele *et al.* (2015). Clinical response in both groups was defined as improvement of organ function (no neurological residues; normalization of kidney function) and independence of red blood cell and platelet transfusions. Results: the median time of TA-TMA onset was 8.2 months (0.5–32.8) after HSCT. Thirty-five of 37 patients (95%) were under treatment with calcineurin-inhibitors or sirolimus at the time the TA-TMA occurred. In all cases, the immunosuppressive drug was stopped promptly. In 28 patients, classical treatment was the primary therapy with a response rate of 52% (including four patients who switched to EC), whereas the response rate to EC treatment was significantly higher with 92% ( $P=0.017$ ). All patients receiving EC showed sufficient blockade of the terminal complement pathway after the second EC application (CH50 < 10%). Despite the increased response rate for EC therapy, there was no difference seen

between these two groups according to overall survival in weeks: classical treatment 9 (95% CI 0–19.3) vs EC treatment 14.9 (95% CI 6.9–22.7)  $P=0.84$ . The main cause of death differed significantly between these two treatment approaches with a therapy-related mortality due to infection with 70% in the EC group during TMA therapy and none seen in the classical treatment group ( $P=0.001$ ). Progressive GvHD was identified as an adverse prognostic factor in both groups ( $P=0.048$  and  $P=0.005$ ). Conclusion: in our analysis, we show that EC shows a significant higher response rate in severe TA-TMA patients compared to the classical treatment approach. However, in both groups the outcome remained very poor. Since most patients presented with advanced, severe TA-TMA, especially in the EC group, we hypothesize that earlier diagnosis and treatment of TA-TMA and more effective prevention and treatment of infections will improve the outcome of patients with this complication. However, randomized studies are essential for comparison of these two treatment strategies to identify patient groups that benefit from a treatment with EC.

**Disclosure of conflict of interest:** None.

### P388

#### **Tocilizumab as an effective treatment in cytokine release syndrome as an early peri-transplant complications in patients subjected to allogeneic stem cell transplantation—proinflammatory/autoimmune patient/donor HLA haplotype life-threatening early allogeneic HSCT complication risk factor hypothesis**

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Cytokine release syndrome (CRS) is classical complication of CAR T cells therapy, but also can be connected with early peri-transplant complications in patients subjected to allogeneic stem cell transplantation. It can be connected with ATG infusion, but also with inflammatory response during perengraftment period (pre-engraftment syndrome and engraftment syndrome) and septic infections. Severity of these complication can differ depending on patient's performance status and therapeutic options from just observation and vigilance to mechanical ventilation need. We would like to present small patient series ( $n=5$ ) subjected to MSD ( $n=1$ ) and MUD ( $n=4$ ) with early transplant-related complications treated with combination of steroids (Dexamethason) and Tocilizumab. In two of them, Tocilizumab was used after second dose of ATG. Both patients present hypotonia with decreased urine output, prompt increase of creatinine level and presence of acute inflammatory parameters CRP, beta2-microglobulin and procalcitonin level, fluid retention and decreased oxygen saturation. In another one patient, these symptoms were connected with PBSC infusion from unrelated donor. In later two patients, we observe almost the same clinical presentation in preengraftment phase. In every of patients infection was ruled out—blood cultures were negative. All these patients were treated with Tocilizumab in a single dose of 8 mg/kg. In all patients, we observed prompt response—normalization of clinical state, renal function, oxygen saturation and decrease of inflammatory factors—CRP, procalcitonin and beta2microglobuline. Discussion: CRS is a rare complication connected with early phase of allogeneic stem cell transplantation. There were no results of treatment with steroids, reduction of a dose of cyclosporine A according to decreased renal function, but all patient completely/fully recovered after single dose Tocilizumab treatment. All our patients were subjected to reduced intensity protocols, what might be a risk factor to develop CRS because non complete depletion of the patient origin monocytes/macrophages active population. We also analyzed other factor connected with CRS in early peritransplant period finding possible connection with

proinflammatory HLA phenotype. It was obvious in the patient one our patients with peri- engraftment phase CRS—he was diagnosed previously with rheumatoid arthritis B27 pos, DR4. In three of five, we have found SLE predisposition in HLA phenotype (DRB1\*1501/DQB1\*0602 or DRB1\*0301/DQB1\*0201), in later one—RA associated HLA antigen DRB1 0101. These patients were analyzed correlating with historical cohort of additional five patients with mortal and another three with very severe early peri-transplant complications and in all we have found the same ‘SLE or RA HLA phenotype’. Because small number of analyzed patients and documented high frequency of these haplotype in population, this is still an opened question is proinflammatory/autoimmune HLA phenotype connected pathogenically with predisposition to develop severe transplant complications and are we able to treat all these patients with combination of steroids with Tocilizumab. Further analysis is needed.

**Disclosure of conflict of interest:** None.

**P389**

**Transplant-associated thrombotic microangiopathy: recognition of patients who would benefit from complement inhibition**

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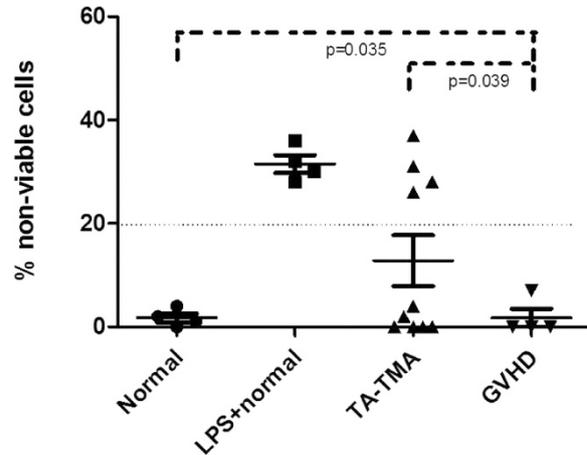
Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe complication post haematopoietic cell transplantation (HCT) leading to high mortality rates. However, outstanding questions regarding its diagnosis, pathophysiology and treatment remain in the literature. Recent studies suggest evidence of complement activation, implicating that complement inhibition may be an effective alternative treatment strategy in refractory patients. Therefore, we hypothesized that increased complement activation can be detected in TA-TMA patients using a functional assay, the modified Ham test. We enrolled consecutive patients with TA-TMA according to the International Working Group criteria from January 2015 to June 2016. As controls, we studied patients with graft-versus-host-disease (GVHD). Complement activation was detected using the modified Ham test, a cell proliferation assay based on the susceptibility of a PNH-like cell line to complement activated serum. Normal human serum was used as a negative control and lipopolysaccharides(LPS)-incubated normal serum as a positive control. All samples were tested in triplicates and twice. We studied 10 TA-TMA patients transplanted from unrelated 8/8 matched (4) or 7/8 mis-matched (3) donors, identical (2) and haploidentical (1) siblings. All patients presented severe acute and/or chronic GVHD. TA-TMA presented at median +109 (9–930) day post-transplant. In the control group, we studied two patients with steroid-sensitive GrII and two with steroid-refractory GrIV acute GVHD. We were able to detect significantly increased complement activation in the serum of TA-TMA compared to GVHD patients ( $P=0.039$ ). Based on previous studies and present controls, percentage of non-viable cells higher than 20% was considered a positive modified Ham test, indicating increased complement activation in four TA-TMA patients. Regarding treatment outcomes, two patients with a negative modified Ham test responded to cyclosporine cessation and steroid administration. Plasma infusion with/without plasma exchange was initiated in seven patients. However, only three of them responded to second-line treatment. The modified Ham test result was significantly increased in refractory patients ( $P=0.048$ ). The terminal complement inhibitor eculizumab was administered in one refractory patient with a positive modified Ham test and renal failure at presentation. Despite delayed initiation (28 days post TA-TMA diagnosis), response was observed after three doses of eculizumab including evidence of reduced hemolysis, schistocytosis and transfusion needs. However, the patient succumbed to

complications of end-stage renal disease (54 days post TA-TMA diagnosis). Among 10 TA-TMA patients, 8 succumbed at a median +215 (73–430) day to transplant-associated complications, related to GVHD and infections from multi-resistant pathogens. TA-TMA is associated with increased morbidity, mortality and severe complications, including GVHD. Unlike GVHD, increased complement activation was observed in a significant portion of TA-TMA patients. Complement inhibition seems an encouraging therapeutic option in these patients. Given the lack of robust functional assays for complement activation, the modified Ham test may be useful for early recognition of patients that would benefit from complement inhibition.

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[P389]



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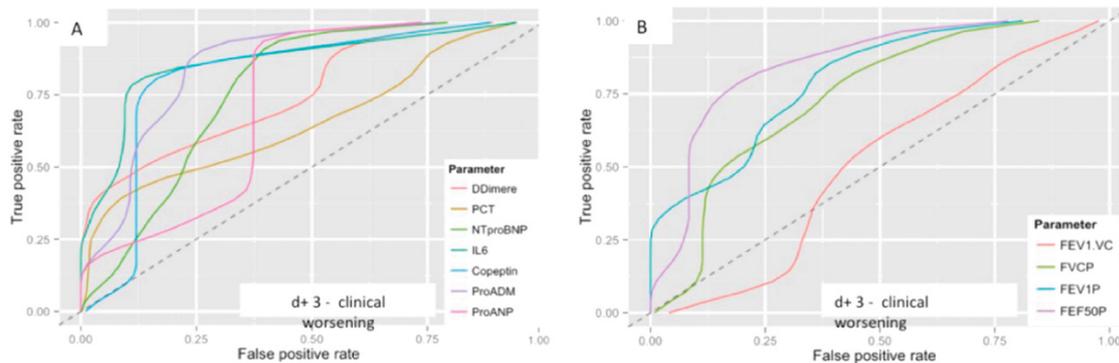
**P390**

**Utility of serial biomarkers and serial pulmonary function test for prediction of ICU-admittance/NRM in allogeneic hematopoietic cell transplantation**

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To evaluate the additional predictive utility of serial Copeptin, MR-proADM, MR-proANP, NT-proBNP, IL-6, PCT, D-Dimer and serial bed-side pulmonary function testing (PFT) for ICU-admittance/NRM in the transplantation course of adult patients undergoing allogeneic hematopoietic stemcell transplantation (allo-HSCT). Single-center, prospective, blinded, thrice weekly analysis of seven biomarkers and serial bed-side PFT in 101 allo-HSCT patients. Everyday vital parameters and the routine laboratory test results were considered in the statistical analysis. Interbiomarker correlations, univariate C-statistics, continuous net reclassification improvement (cNRI), multivariate logistic regression analysis, multivariate ROC-analysis and odds ratios were calculated. Time window for statistical analysis was day +3 (to avoid ATG-confounder) until

**Figure 1:****ROC-curves to predict ICU-admission and/or NRM in adult patients undergoing allo-HSCT**

**A** biomarkers and **B** pulmonary function parameters

in the timewindows d+3 until clinical worsening defined as more than  $\pm 5$ kg or body temperature  $\geq 38.5$  or shock-index  $\geq 1$ .

ICU-admittance regarded only in case of mechanical ventilation and/or vasopressor therapy and/or contiuous veno-venous hemofiltration to focus the most jeopardized patients or in-hospital non-relapse mortality.

overt clinical deterioration defined as: body temperature  $> 38.5$  °C and/or body weight +5 kg and/or a shock index  $> 1$ . None of the basic demographic parameters correlated significantly with the patient's outcome. Inter-biomarker correlation coefficients: 0.002–0.82. Highest AUC-values: IL-6 (0.87), MR-proADM (0.86), FEF50% (0.86) and Copeptin (0.82). In contrast the AUCs of CRP (77.3), WBC (75.6) and creatinine (0.63) were low. Multivariate logistic regression selects the combination of MR-proADM+D-Dimer as the best prediction model. Highest univariate odds ratios for one unit MR-proADM (18.1) and creatinine (7.9), highest odds ratios for the interquartile range Copeptin (5.9), MR-proADM (3.5) and D-Dimer (3). High reclassification numbers (cNRI) Copeptin (0.9) and D-Dimer (0.92), compared to index test of the best standard laboratory model (CRP+platelets+GGT) plus patient's age. Multivariate odds ratio if Copeptin is added to the baseline model is 4.2. Multivariate AUC raised from 0.84 (best baseline model) to 0.92 for both MR-proADM+D-Dimer or Copeptin+D-Dimer. Serial PFT was often not tolerated by several patients with mucositis. In the patients who tolerated the PFT examinations, the ventilation parameters were of added predictive utility with high AUC and cNRI values; and low odds ratios when the ventilation parameters were near normal. Most of the standard laboratory parameters correlated hardly with the patient's outcome. Serial analysis of IL-6, MR-proADM, Copeptin and D-Dimer provides additional predictive information in the transplantation course of adult patients undergoing allo-HSCT. These biomarkers open the door for very-early risk stratification before clinical worsening.

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#### P391

#### Very severe hepatic sinusoidal obstruction syndrome continues to be an important cause of mortality after allo-HSCT

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Hepatic sinusoidal obstruction syndrome (SOS), previously named hepatic veno-occlusive disease (VOD), is a potentially fatal complication that might occur after HSCT. Recently, EBMT has proposed a new definition for diagnosis and new severity criteria for SOS in adults (Mohty *et al.* *BMT* 2016). This proposal includes, along with the 'classical SOS' (cases diagnosed before day +21), the new type 'late onset SOS' (cases diagnosed afterwards). New EBMT criteria for severe grading classify cases of SOS into four grades (mild, moderate, severe, and very severe). The aim of this retrospective study is to analyze the cases of severe/very severe, both classical and late onset SOS, occurred in our Unit during the most recent period of time. We studied the last 100 pts, with a minimum follow-up of 100 days, who underwent allo-HSCT in our center (November 2014–August 2016). 56 pts were male and 44 female. Median age was 53 years (range: 7–69). Baseline diseases were: acute leukemias (54), lymphoproliferative disorders (17), myelodysplastic syndromes (12), chronic myeloproliferative diseases (7), multiple myeloma (5), and bone marrow failures (5). Donor was unrelated in 57 cases, and related in 43 (including 18 haplo-identical). Conditioning regimen was: busulphan-based (70), melphalan-based (13), TBI-based (8), and others (9). All patient received prophylactic

ursodeoxycholic acid. Progenitors source was PB in 89, and BM in 11. Five patients developed severe/very severe SOS (5% incidence); 3 were classical (at days +8, +11 and +20), and 2 were late onset (at days +34 and +44) (see Table 1). Four cases had received conditioning with a busulphan (iv)-based regimen (doses from 6.4 to 12.8 mg/kg), and one case with TBI plus cyclofosamide at high doses. All cases presented with right upper quadrant pain, jaundice, ascites, weight gain, hiperbilirrubinemia, and renal function impairment. All but one had increased transaminases. The five cases were treated with Defibrotide, in spite of which all of them died. Considering that overall day +100 mortality was 9%, severe/very severe SOS was the most important cause of death of the series.

[P391]

Patient	Age (years)	Disease	Donor	HLA	Onset	Cause of death
1	54	Myelofibrosis	URD	09/10	Day + 8	Bleeding
2	55	Hodgkins disease	Haplo-id	--	Day + 11	MOF
3	32	AML	Haplo-id	--	Day + 20	MOF
4	35	ALL	URD	09/10	Day + 34	MOF
5	58	ALL	URD	10/10	Day + 44	MOF

Although milder forms of SOS might resolves within weeks, the most severe forms are still associated with a very high mortality rate. Prophylaxis with Defibrotide (the drug currently licensed for treatment) for high-risk patients has not been sufficiently studied yet. Therefore, a high index of suspicion, early detection and early therapy are the only ways to try to reduce mortality due to SOS in the HSCT setting.

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### P392

Previously published

## Conditioning regimen

### P393

#### Alemtuzumab-conditioned unmanipulated stem cell top-up is a safe and effective intervention for poor graft function following allogeneic HPC transplantation

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Secondary poor graft function (sPGF) complicates up to 15% allogeneic HCTs, and is associated with increased mortality and poor quality of life due to recurrent infections and the need for ongoing blood product support. Potential interventions include a second allograft using further conditioning, however many patients with sPGF have a reduced performance status and are at an increased risk of complications from this procedure. Unconditioned haematopoietic progenitor cell (HPC) top-ups are associated with a high risk of GVHD if unmanipulated cellular products are used. CD34+ selection offers an attractive alternative, but incurs a loss of up to 20% HPCs and is an expensive procedure, unavailable to many centers internationally. Alemtuzumab, a monoclonal anti-CD52 antibody, is routinely used in allogeneic transplant conditioning in the UK to prevent GVHD. We report the results of a retrospective study examining the efficacy of alemtuzumab conditioned HPC top-ups for sPGF. Data pertaining to patients

who had undergone a second infusion of HPCs from their original donor were identified from our hospital-specific ProMiSe database. Those who met the criteria of sPGF defined as  $\geq 2$  of hemoglobin  $20 \times 10^9/L$  without support. 10 patients (4 pediatric, 6 adult) who underwent initial allogeneic transplants for malignancy (8) or bone marrow failure (2) received an alemtuzumab conditioned HPC top-up for sPGF at our center 2005–2016. The diagnosis of sPGF was made at a median 2.6 months post allograft (range 2–60) with trilineage cytopenias in 7 patients and bilineage cytopenias in 3 patients. All patients had received transplants from 10/10 (7 patients) or 9/10 (3 patients) matched unrelated donors. The median interval between initial transplant and top-up was 187 days (range 87–1853), and a median CD34 dose of  $2.7 \times 10^6/kg$  recipient weight (range 0.3–7.63) was infused. 90% patients achieved haematological improvement (HI) at a median 20 days post-top-up (range 13–179), with the only failure to achieve HI seen in the patient who had received the lowest CD34 dose ( $0.3 \times 10^6/kg$ ). One patient developed grade I aGVHD post top-up but no grade II–IV aGVHD was observed. 1 year OS was 80% and 2 year OS 60% following HPC top-up. 3 deaths occurred due to infection at 1, 5 and 23 months post top-up, and one due to relapse of a prior non-haematological malignancy. 9 patients had an aplastic or hypocellular BM trephine pre-top up, which was repeated at 100 days post top-up in 6 patients, of whom 5 had a normocellular BM trephine, while 1 remained hypocellular. Alemtuzumab conditioned HPC top-up appears an effective intervention for sPGF with results comparable to those of CD34 selected top-ups, and therefore represents a feasible alternative. Larger studies are needed to exclude complications including viral reactivation and to investigate immune reconstitution following this procedure.

**Disclosure of conflict of interest:** None.

### P394

#### Autologous haematopoietic stem cell transplantation using BEAM ± rituximab for relapsed diffuse large B cell lymphoma in the rituximab era: a 10-year follow-up of a single transplant center in Singapore

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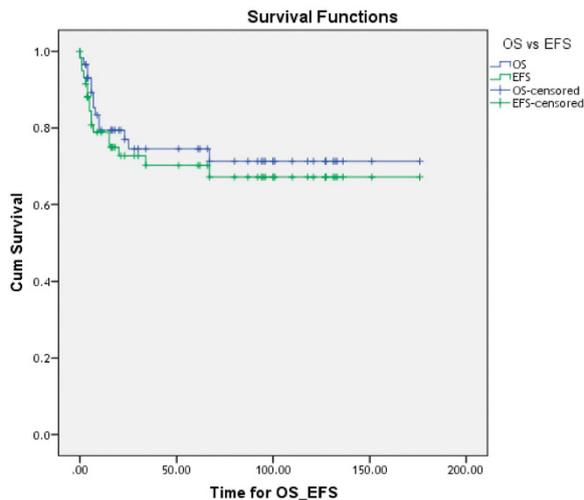
High dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) has shown to improve outcome in patients with relapsed/refractory diffuse large B cell lymphoma (DLBCL). In the Rituximab era, the benefit of ASCT has been debatable as prior study (CORAL study) has shown that patients who received R-CHOP as induction chemotherapy & responded to salvage chemotherapy had a poorer outcome following ASCT compared with those who received CHOP alone. In addition, it remains unclear whether addition of Rituximab to standard high dose BEAM regimen provides any additional benefit. We retrospectively analyzed 63 DLBCL patients receiving high dose BEAM ( $n=27$ ) or Rituximab +BEAM (R-BEAM) ( $n=36$ ) followed by ASCT for relapsed/refractory DLBCL since 2002. All patients who received CHOP ( $n=15$ ) ± Rituximab ( $n=48$ ) as first line therapy and who received  $\leq 2$  lines of salvage chemotherapy before ASCT were analyzed. Rituximab was given at the dose of 375 mg/m<sup>2</sup> on day +1 and +8 of ASCT. Twenty-two (81%) patients in BEAM group and all the patients (100%) in R-BEAM group received Rituximab-based salvage chemotherapy prior to ASCT. The 10-year overall survival (OS) was 71% and event-free survival (OS) was 67% for the whole cohort. R-CHOP induced patients did not fare any worst after ASCT than CHOP induced patients (10 year OS 73 vs 68 %;  $P=0.91$ ). There was a trend towards better survival in patients with pre-transplant disease free interval (DFI)  $> 12$  months compared to those with DFI 500/ $\mu$ l time was 10 days and 11 days, respectively. Median platelet recovery ( $> 20,000/\mu$ l) time was 17 days and 11 days, respectively ( $P=0.11$ ). Ten year OS (67% R-BEAM vs 77%

BEAM,  $P=0.38$ ) and EFS (65% R-BEAM vs 73% BEAM,  $P=0.28$ ) were also comparable between both groups. HDT with BEAM and ASCT remains beneficial for patients with relapsed/refractory DLBCL. It should be offered to all patients who respond to salvage chemotherapy with the expectation that they fare no worse than patients who do not receive Rituximab in the induction chemotherapy. Addition of Rituximab following the standard BEAM for HDT and ASCT does not compromise haematopoietic recovery, but does not result in improved outcome in our study. Prior use of Rituximab during first-line or salvage therapy in most of the patients of R-BEAM group might have negated the beneficial effect of R-BEAM over BEAM.

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[P394]



Overall survival (OS) and Event free survival (EFS) of whole cohort

**Disclosure of conflict of interest:** None.

**P395**

#### Autologous peripheral stem cell transplantation with R/BEAM protocol after modified R/IDARAM/ RT in DLCL with CNS involvement: a single-center experience

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Central nervous system (CNS) involvement of diffuse large cell lymphoma (DLCL) is a sign of poor prognosis and there is no standard treatment regimen (1). In this study, We aimed to develop a CNS targeted chemotherapy regimen, which has lower toxicity and higher complete remission rates, in combination therapy. Eight patients with secondary CNS lymphoma (SCNSL) and two with primary CNS lymphoma (PCNSL), followed between the years 2010 and 2015, were included in the study, retrospectively. The patients were histologically diagnosed with biopsy and underwent autologous stem cell transplantation (APKHT). All patients were treated with R-IDARAM/ RT (radiotherapy)/subsequently autologous stem cell transplantation (APSCT) with R-BEAM protocol. The R-IDARAM regime consists of the following substances: Rituximab 375 mg/m<sup>2</sup>, 50 cc/h infusion, day 1; cytosine arabinoside 1.0 gr/m<sup>2</sup> i.v., 1 h infusion, days 2 and 3; Dexamethasone 100 mg, 12 h infusion, days 2, 3 and 4;

Idarubicin 10 mg/m<sup>2</sup> i.v., 15 min infusion, days 2 and 3; Methotrexate 3 gr/m<sup>2</sup> (2 gr/m<sup>2</sup> at >45 years old-patients), 6 h infusion, day 4; and cytosine arabinoside 70 mg plus Methotrexate 12 mg, intrathecally, days 2 and 8. The patients included seven males and three females. The median age was 44 years (range: 23–67). Six SCNSL patients were diagnosed in the application and two of them were diagnosed during R-CHOP chemotherapy (CT) protocol. Five patients (57%) were stage IVB, and the others (43%) were stage IIIB at diagnosis. After two or three chemotherapy cycles, patients were mobilized with growth factor support and median 5.510<sup>6</sup> cells per kg (range: 4–8) stem cells were collected. Then, at a dose of 3600–4140 CGY cranial RT was administered for 14 days. After the third cycle of R/IDARAM, the state of remission was evaluated by cranial MRI and lumbar puncture (LP). All patients achieved complete remission. Neutrophil engraftment occurred at a median of 12 days (range: 10–18) and platelet engraftment occurred at a median 16 days (range: 11–21). After APKHT, three patients relapsed and died at the fourth, ninth, and thirteenth months. Grade I–II manageable neurological toxicity occurred in two patients. The median follow-up time was 24 (range: 2–74) months. The five-year overall-survival (OS) was 63%. Serious signs of infection were not observed in patients during transplantation. In PCNSL and SCNSL, a standard treatment regimen has not yet been found. APSCT with R-BEAM following modified R/IDARAM/RT is a curative and applicable therapeutic regimen with low toxicity, which can provide high rates of long-term survival and disease-free survival.

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**Disclosure of conflict of interest:** None.

**P396**

#### Carfilzomib, bendamustine, and melphalan (CBM) conditioning prior to autologous hematopoietic stem cell transplantation for multiple myeloma is feasible and safe

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Despite the advent of novel therapies, autologous hematopoietic stem cell transplantation (AHSCT) following melphalan (M)-based conditioning remains the standard of care for patients with multiple myeloma who are eligible. Still, the majority of patients experience disease progression and ultimately succumb to their disease. We hypothesize that integrating novel agents in the conditioning is feasible and safe and may increase complete remission rates and overall survival. We completed a phase I, dose escalation study of carfilzomib (C) added to a backbone of bendamustine (B) and melphalan. All patients received a fixed dose (20 mg/m<sup>2</sup>) of C on days (d) –29, –28, –22, –21, –15 and –14. In addition, patients were conditioned as described in Table 1. Due to dose-limiting toxicity in cohort 2, the study was amended after the first 6 patients. Subsequently, the dose of M was reduced to 140 mg/m<sup>2</sup> and the d +6 dose of C was omitted, per oversight of a data safety monitoring board. Fifteen patients were enrolled, 9 males and 6 females. Median age was 56 years (39–68). Performance status was ≥80% (KPS) in all patients. Per the International Staging System (ISS), 3 patients had stage I disease, 5 had stage II, 6 had stage III, and 1 had unknown staging. Three patients had high-risk cytogenetics: 2 with t(4;14) and 1 with deletion 17p. Four patients had undergone a prior AHSCT. Disease status at enrollment was stable disease (SD) (n=3), partial response (PR) (n=8), or very good partial response (VGPR) (n=4). Median CD34+ cell dose infused was 3.11 × 10<sup>6</sup>/kg (2.23 – 6.92 × 10<sup>6</sup>). Median follow-up was 18.2 months (1.4–28.7). All fifteen patients are evaluable

Cohort	Patients (n)	C (mg/m <sup>2</sup> )	B (mg/m <sup>2</sup> )	M (mg/m <sup>2</sup> )
1	3	15 d -2, -1, +5, and +6	120 d -2 and 100 d -1	100 d -2 and -1
2	3	20 d -2, -1, +5, and +6	120 d -2 and 100 d -1	100 d -2 and -1
2B	3	20 d -2, -1, and +5	120 d -2 and 100 d -1	140 d -1
3B	7	27 d -2, -1, and +5	120 d -2 and 100 d -1	140 d -1

for engraftment. Median time to neutrophil engraftment was 12 d (11–15). One patient died before achieving platelet engraftment. For the remaining patients, median time to platelet engraftment was 16 d (12–20). Non-hematologic toxicities included grade 3 acute mucositis ( $n=1$ ), lower GI complications ( $n=7$ ), electrolyte disturbances ( $n=7$ ), transaminase elevation ( $n=1$ ), renal insufficiency ( $n=1$ ), atrial fibrillation ( $n=1$ ), hypoxia ( $n=1$ ), prolongation of the QTc interval ( $n=1$ ), and grade 4 acute sepsis ( $n=2$ ), including 1 death (cohort 2) on d +44. Eight patients went on to receive maintenance therapy: 3 with bortezomib, 3 with lenalidomide, and 2 with lenalidomide, dexamethasone, and C. Post-transplant disease status was assessed per protocol by SPEP, SPIF, serum free light chains, and light chain ratio. Twelve patients were evaluable on d +100. Two patients had SD, 7 had VGPR, and 3 had complete response (CR). Eight patients were evaluable on d +365. Two patients had progressive disease, 1 had PR, 3 had VGPR, and 2 had CR. The combination of CBM prior to AHST appears feasible, with manageable toxicities, at the doses described in cohort 3B. A prolonged follow-up and a phase II study are warranted to determine response rates and long-term outcomes.

**Disclosure of conflict of interest:** None.

### P397

#### Previously published

### P398

#### Comparable safety profile of BeEAM (bendamustine, etoposide, cytarabine and melphalan) and BEAM as conditioning before autologous HCT

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BEAM (carmustine, etoposide, cytarabine, melphalan) is the most frequently used high-dose chemotherapy regimen for patients with lymphoma referred for autologous hematopoietic cell transplantation (autoHCT). In recent years a novel conditioning protocol containing bendamustine instead of carmustine (BeEAM) has been proposed in order to potentially increase the efficacy. So far, however data on its safety are limited. The aim of this study was to retrospectively compare the safety profile of BEAM and BeEAM based on single center experience. 174 consecutive patients with lymphoma treated with BEAM and 63 patients treated with BeEAM between year 2011 and 2016 were included in the analysis. The median age was 47 (19–69) years and 46 (22–73) years, respectively ( $P=NS$ ). Clinical characteristics of both groups were comparable. Patients with Hodgkin's lymphoma constituted 49% in the BEAM group and 40% in the BeEAM. Among those with non-Hodgkin lymphoma the diagnosis of DLBCL predominated. BEAM treatment consisted of carmustine 300 mg/m<sup>2</sup> on day -6, etoposide 400 mg/m<sup>2</sup>/d on days -5 to -2, cytarabine 400 mg/m<sup>2</sup>/d on days -5 to -2, and melphalan 140 mg/m<sup>2</sup> on day -1. In the BeEAM regimen carmustine was substituted by bendamustine administered on days -7, -6 at the total dose of 400 mg/m<sup>2</sup> i.v. Peripheral blood was used as a source of stem cells. CD34+ cell dose was 5.1 (1.5–42.6) × 10<sup>6</sup>/kg in the

BEAM group and 4.1 (2–16.8) × 10<sup>6</sup>/kg in the BeEAM group ( $P=NS$ ). Time to engraftment and the rates of adverse events up to day +100 after autoHCT were the study endpoints. All patients engrafted in both study groups. Median time to neutrophil > 0.5 × 10<sup>9</sup> recovery was 11 (7–37) days after BEAM and 10 (7–12) days after BeEAM ( $P=0.13$ ). Median time to achieve platelet count > 50 × 10<sup>9</sup> was 13 (7–44) days and 14 (7–33) days, respectively ( $P=0.29$ ). Two patients died without progression before day +100 in the BEAM group, both due to bacterial infections. No early deaths were reported in the BeEAM group. The rates of grade 3 or 4 adverse events were comparable (see: Table 1). Administration of bendamustine instead of carmustine as part of conditioning does not affect engraftment as well as toxicity profile of the regimen. Therefore BeEAM may be safely used in patients with lymphoma undergoing autoHCT. Its efficacy requires evaluation in prospective studies focused on homogenous patient populations.

[P398]

**Table 1. Toxicity of BeEAM as compared to BEAM**

Grade 3 or 4 adverse events	BEAM (n=174)	BeEAM (n=63)	p
Febrile neutropenia	39 (22%)	14 (22%)	0.98
Pneumonia	4 (2%)	1 (2%)	0.74
Nausea	24 (14%)	7 (11%)	0.59
Vomiting	8 (5%)	2 (3%)	0.63
Mucositis	11 (6%)	5 (8%)	0.66
Diarrhea	8 (5%)	4 (6%)	0.59

**Disclosure of conflict of interest:** None.

### P399

#### Comparison of low dose total marrow/total lymphoid irradiation (TMI/TLI) and TBI in haploidentical transplant platform: a retrospective analysis

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The Baltimore group reported a low dose TBI-based non-myeloablative conditioning regimen followed by T cell replete bone marrow, with post-transplantation cyclophosphamide (PT-Cy) to control GVHD and graft rejection. Based on the fact that in our facility conventional low dose TBI was not available, we wanted to explore whether TMI/TLI could be a potential substitute. The aims of our study was to explore if TMI/TLI can be considered an effective substitute of TBI in terms of OS, PFS and NRM. Retrospective analysis was applied in 159 cases of haploidentical HSCT from April 2009 to October 2016. All patients underwent Baltimore conditioning associating fludarabine (30 mg/m<sup>2</sup>/day) day -6 to -2, Cy (14.5 mg/kg/day) on days -6 and -5, and TBI 2 Gy in 135 patients and TMI/TLI 2 Gy

in 24 patient at day -1. Unmanipulated bone marrow graft was infused at day 0. Postgrafting immunosuppression consisted of Cy (50 mg/kg/day) on day +3 and +4, and mycophenolate mofetil for 30 days, and tacrolimus or cyclosporine. No differences between the two groups was observed in term of age, gender diagnosis, disease status and donor type. 93% of patients engrafted in both arm (23/24 and 125/135). In TBI cohort vs TMI/TLI cohort, the median time to ANC >500/ $\mu$ L and platelet recovery >20 000/ $\mu$ L was not different (22 and 27 days vs 20 and 27.5 days,  $P=0.14$  and 0.32, respectively). In all TMI/TLI evaluable patients, full chimerism was observed at days +30. After a median follow-up of 17 months in TMI/TLI cohort and 34 months in TBI arm, 1-year NRM was 25.9% and 13.9% ( $P=0.16$ ), respectively. The 1 years OS and PFS were not statistically different in the two groups 70% vs 57.1%,  $P=0.12$  and 62.5% vs 48.1%,  $p 0.09$ , respectively). The 1-year relapse incidence was 26% in TMI/TLI group and 23.6% in TBI group,  $P=0.61$ . No difference in incidence of both aGVHD and cGVHD was observed between the two groups. This retrospective analysis suggests that TMI/TLI could be considered an effective substitute of low dose TBI, with a sufficient degree of immunosuppression of recipient, allowing engraftment and full chimerism. The GVHD both acute and chronic as well as the 1-y NRM were not different. **Disclosure of conflict of interest:** None.

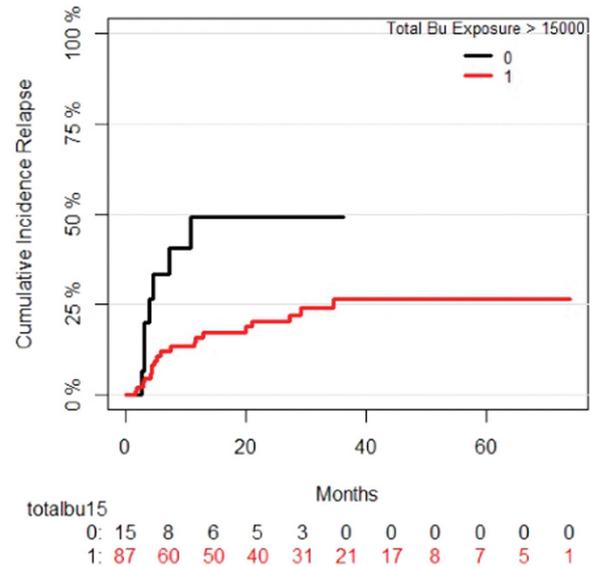
#### P400

##### Comparison of the BeAM conditioning regimen and the BEAM conditioning regimen in the autologous transplantation for HL and NHL

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The BEAM has established itself as a standard of care conditioning regimen in the autologous lymphoma HSCT setting for most transplant centres in Europe. Yet however various other regimens are being compared with it in order to achieved better safety profile, better OS and DFS, in order to improve results with chemoresistant and unfavourable patients. One such regimen is BeAM (bendamustine, etoposide, cytarabine, melphalan). We aimed to compare the efficacy of the BEAM and BeAM conditioning regimens and to compare their myelotoxicity profile. We evaluated retrospectively 114 adult patients (mean age 41.1197 with SD 11.12404), receiving auto-HSCT at the National Specialized Hospital for Active Treatment of Hematological Diseases in Sofia, Bulgaria for relapsed/refractory HL or NHL (of them MH - 57, DLBCL - 26, PMBCL - 15, FL - 3, LBL - 3, PTCL-NOS - 3, AITL - 2, ALCL - 2, MCL -2, MZL -1) for the period from 1.01.2013 to 1.07.2016 with a follow-up of patients up to 1.11.2016. Ninety-two of the patients received the BEAM (as previously described - BCNU 300 mg/m<sup>2</sup> i.v. day -6, etoposide 200 mg/m<sup>2</sup> i.v. days -5 to -2, cytarabine 400 mg/m<sup>2</sup> i.v. days -5 to -2, and melphalan 140 mg/m<sup>2</sup> i.v. day -1) regimen and 22 received BeAM regimen (bendamustine on days -7 and -6 (160 mg/m<sup>2</sup>); cytarabine, 400 mg/m<sup>2</sup> intravenously daily, from day -5 to day -2; etoposide, 200 mg/m<sup>2</sup> intravenously daily, from day -5 to day -2; and melphalan, 140 mg/m<sup>2</sup> intravenously on day -1). The overall survival at the second and third years of follow-up (OS-2, OS-3) and DFS at the third year, the CR rates and the average time periods to hematological recovery, were compared. The OS at 2 and 3 years, respectively, was 86.1% and 86.1%, for BeAM and 78.8% and 71% for BEAM, the DFS at 3 years was 76.4% for BeAM and 73.2% for BEAM, provided that the differences did not have statistical significance ( $p 0.851$  for OS and  $p 0.890$  for the DFS). The CR rate was 63.63% in the BeAM group versus 50% in the BEAM group. From the patients who received autologous HSCT in stable disease or progression pre-transplant status (chemoresistant patients), 22.72% of the patients receiving BeAM achieved CR at the first post-transplant evaluation versus 10.86% respectively for the BEAM group. The mean time to hematological recovery for

neutrophils was 11.1765 $\pm$ 4.91471 days (BeAM) versus 10.2469 $\pm$ 3.56216 days (BEAM) and 12.6471 $\pm$ 6.04091 days (BeAM) versus 11.1235 $\pm$ 2.52677 days (BEAM) for platelets. BeAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better response rates in chemoresistant patients. [P400]



**Disclosure of conflict of interest:** None.

#### P401

##### Conditioning regimen with cisplatin (PEAM) versus carmustine-BCNU (BEAM) followed by autologous haematopoietic stem cell transplantation in lymphoma

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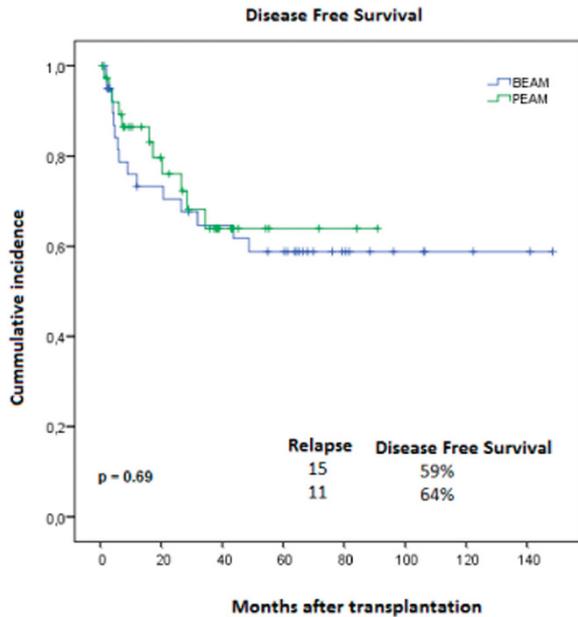
Autologous hematopoietic stem cell transplantation (ASCT) is widely used as a consolidation therapy in aggressive non-Hodgkin's lymphoma (NHL) and recurrent or refractory classic Hodgkin's lymphoma (HL). In Mexico, the use of Carmustine (BCNU) in the conditioning regimen of these patients is limited due to the lack of access to the drug and its high costs. This study aims to compare results in terms of toxicity, disease-free and overall survival between a group of patients treated with the standard regimen BEAM and another group treated with a scheme in which carmustine was replaced by cisplatin (PEAM regimen). A comparison of two groups with lymphoma was performed and the clinical aspects of Cisplatin 100 mg/m<sup>2</sup> d -4, etoposide 750 mg/m<sup>2</sup> d -4, -3, cytarabine 800 mg/m<sup>2</sup> d -4, -3, -2 and melphalan 140 mg/m<sup>2</sup> d -4 (PEAM) and carmustine-BCNU-, etoposide, cytarabine and melphalan (BEAM). Regimens were evaluated retrospectively. Between January 2004 and December 2015, 84 patients (NHL and HL) underwent high-dose chemotherapy with PEAM (N=44) or BEAM (N=40), followed by ASCT at the National Cancer Institute of Mexico City. The median age was 30.5 years (16-62). The characteristics were well balanced between the two groups. The mean time for neutrophil grafting (>500 per mm<sup>3</sup>) was significantly slower with BEAM than with PEAM (12 vs 11 days,  $P=0.001$ ), hospitalization time was longer with BEAM compared to PEAM (25 vs 22,  $P=0.015$ ). On the other hand, proportion of patients who require red blood cell

transfusion was significantly higher in BEAM group (58%) versus PEAM group (20%) ( $P < 0.001$ ), but total amount of platelet transfusion did not differ between groups. About the toxicity, BEAM patients had significantly more frequent incidence and severity of nausea/vomiting (95% vs 53.8%) and diarrhea (61.5% vs 90%) compared to PEAM ( $P < 0.01$ ). No significant differences were observed in incidence of mucositis ( $P = 0.65$ ). At the moment of the analyses, 75% of patient of the PEAM group were in complete response versus 59% of the patients treated with BEAM, but it did not represent a significant difference. Disease-free survival and 5-year overall survival in the PEAM vs BEAM scheme were similar with 64% vs 59% ( $P = 0.69$ ) and 84% vs 76% ( $P = 0.35$ ) respectively but with less toxicity using the PEAM scheme. PEAM regimen is not inferior scheme compared with BEAM, because it shows similar outcomes in disease-free survival and overall survival. Additionally, PEAM is a well-tolerated regime and BEAM scheme was associated with greater gastrointestinal toxicity such as nausea, vomiting and diarrhea, also greater hematology toxicity such as more requirement of red blood cell transfusion.

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[P401]



Disclosure of conflict of interest: None.

#### P402

### Cumulative busulfan exposure is associated with relapse following busulfan and cyclophosphamide myeloablative allogeneic stem cell transplantation for acute myeloid leukaemia

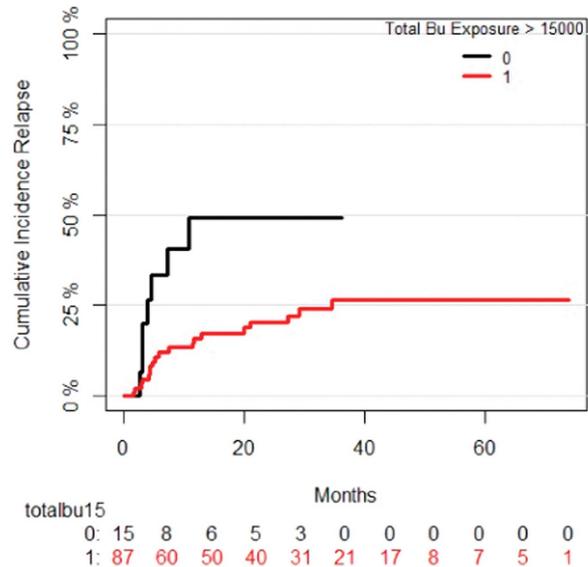
E Wong, D Kliman<sup>1</sup>, M Chau<sup>2</sup>, J Szer<sup>2</sup>, C Nath<sup>1</sup>, P Shaw<sup>1</sup>, D Ritchie<sup>2</sup>, D Gottlieb<sup>1</sup> and A Bajel<sup>2</sup>

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The optimal busulfan exposure to reduce disease relapse in adult patients with acute myeloid leukaemia (AML) undergoing busulfan/cyclophosphamide myeloablative allogeneic stem cell transplant (alloSCT) is poorly defined. We retrospectively analysed busulphan pharmacokinetics (PK) and

outcomes of patients who underwent busulfan/cyclophosphamide conditioned alloSCT for AML from 2010 to 2016. Busulfan was administered intravenously over 5 days (1.6 mg/kg/d for 2 days followed by 3.2 mg/kg for 3 days). Peripheral blood was obtained for busulfan PK after the first dose. Subsequent doses of busulfan were decreased if daily busulfan exposure (area under the curve; AUC) was anticipated to exceed 5000  $\mu\text{M}$  per min/day. Cyclophosphamide was dosed at 120 mg/kg. The primary outcome was the cumulative incidence of relapse (CIR) accounting for non-relapse mortality (NRM) as a competing risk. Independent variables analysed included age, sex, cytogenetic risk group, disease risk index (DRI), donor type, stem cell source, T-cell depletion, and cumulative busulfan AUC (cumAUC) calculated as previously described.<sup>1</sup> 102 patients were included with median follow-up 34 months. Median age at transplant was 41 years (range 18–59). 26 (25%) patients had adverse cytogenetics. 53 patients (52%) received BuCy while the remainder received CyBu conditioning. 49 patients (48%) received grafts from matched unrelated donors, and 46% received T-cell depletion. 38% of patients developed acute GVHD including 25% with grade 2–4 acute GVHD. Of the patients alive and without disease relapse at day 100, 56% developed chronic GVHD. OS at 1 year was 78.1%. The median cumAUC was 19 318  $\mu\text{M}$  per min (range 11 401–27 144  $\mu\text{M}$  per min). 87 patients (85%) had a cumAUC > 15 000  $\mu\text{M}$  per min. 27 patients (26%) had reductions in their busulfan dose on account of their initial busulfan level. 4 patients had their busulfan doses increased at their physicians' discretion, although this was not protocolised. On univariate analysis, cumAUC > 15 000  $\mu\text{M}$  per min was associated with lower CIR (HR 0.34;  $P = 0.019$ ). The CIR at 1 year in patients with cumAUC > 15 000  $\mu\text{M}$  per min was 16.0% compared with 49.2% in patients with cumAUC < 15 000  $\mu\text{M}$  per min (Figure 1). T-cell depletion was also associated with increased CIR (HR 3.3;  $P = 0.0049$ ). Patient age, sex, cytogenetic risk, DRI and graft type were not significantly associated with CIR. On multivariate analysis, cumAUC > 15 000  $\mu\text{M}$  per min remained significantly and independently associated with lower CIR (HR 0.38;  $P = 0.036$ ). CumAUC was not associated with NRM, RFS, OS, or the incidence of acute or chronic GVHD. Figure 1. Cumulative incidence of relapse in patients stratified by total busulfan exposure.

[P402]



Cumulative busulfan exposure > 15 000  $\mu\text{M}$  per min is independently associated with reduced relapse following busulfan/cyclophosphamide alloSCT for adults with AML. These findings support further evaluation of the optimal

busulfan exposure to reduce AML relapse in a prospective clinical trial, whereby patients could be randomised to target cumAUC >15 000 µmol per min versus standard practice.

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**Disclosure of conflict of interest:** None.

**P403**

**CyBu compared to BuCy conditioning results in lower liver toxicity but does not affect other stem cell transplant characteristics**

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Hematopoietic stem cell transplant with busulfan and cyclophosphamide (BuCy) based conditioning has a relatively high incidence of liver toxicity and sinusoidal obstruction syndrome (SOS). Busulfan and cyclophosphamide metabolites share the same glutathione conjugation in the liver metabolism. A small number of studies addressed different sequence of both drugs BuCy vs CyBu during conditioning. Differences in liver toxicity, SOS, transplant related mortality (TRM), relapse incidence (RI) and overall survival (OS) were reported favoring CyBu conditioning. We decided to address the above issues at the UMC Ljubljana, Slovenia. This was a retrospective study following patients with myeloid malignancies (AML, MDS, MPN) with BuCy (*n*=30) and CyBu (*n*=14) conditioning through a three year period in a single institution. Primary endpoint was detecting difference in liver toxicity by measuring levels of liver enzymes. Secondary endpoints were incidence of SOS, difference in TRM, RI and OS. Patients characteristics between groups at the time of the transplant did not differ significantly. We observed significantly higher liver toxicity through elevated bilirubin and ALT in the BuCy 73.3% than CyBu 64.3% patient group (Picture 1). The highest probability of liver toxicity was around D0 in the BuCy group and in the second week after the transplant in the CyBu group. The incidence of SOS, TRM and RI were comparable between the groups. There was no difference in OS between the patient groups during the 40-month follow-up. BuCy conditioning for hematopoietic stem cell transplant causes higher incidence of liver toxicity compared to CyBu conditioning. There is no difference in SOS frequency, TRM, RI and OS between BuCy and CyBu conditioning. Prospective controlled comparison would be needed for further study of the subject.

**Disclosure of conflict of interest:** None.

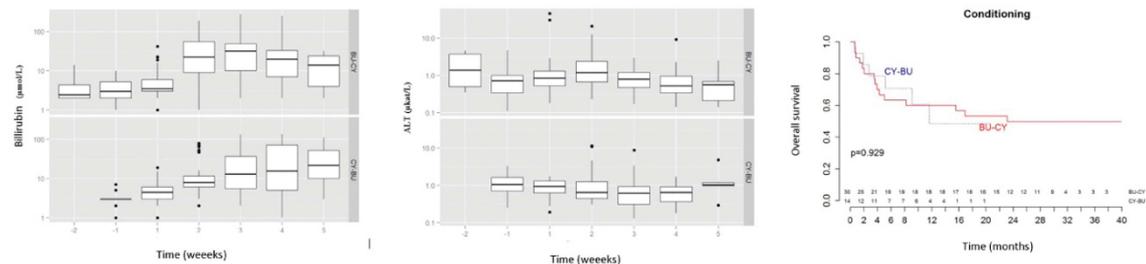
**P404**

**Early monocyte recovery is associated with better overall survival after busulfan containing myeloablative conditioning allogeneic hematopoietic cell transplantation in patients with acute myeloid leukemia**

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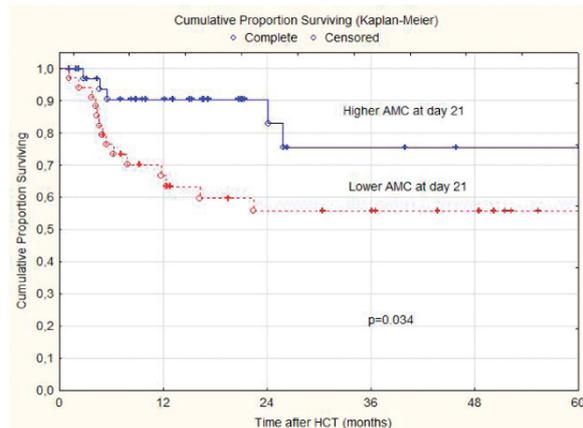
The outcomes of allogeneic hematopoietic cell transplantation (alloHCT) in acute myeloid leukemia (AML) depend on different patient-, disease- and transplant related factors, including the dose and combination of agents used for conditioning. The aim of the study was to analyze the outcomes of alloHCT in patients with intermediate or high risk AML according to Disease Risk Index (DRI) who received myeloablative conditioning consisted of intravenous busulfan (9.6–12.8 mg/kg) combined with cyclophosphamide (120 mg/kg) or fludarabine (150 mg/m<sup>2</sup>) between 2010 and 2016 in our institution. The published data indicate that the combination of busulfan (Bu) and fludarabine (Flu) seems to have more favorable toxicity profile than combination of Bu and cyclophosphamide (Cy), so BuCy regimen has been substituted with BuFlu as the myeloablative conditioning for AML patients in our institution practice since 2014. We evaluated the influence of type of regimen on transplant outcomes along with the impact of other potential prognostic factors, including age of patient, DRI, donor type, HLA and gender mismatches, stem cells source, and lymphocyte and monocyte recovery. The study group consisted of 71 AML patients, median age 37 years (range: 19–59), classified as intermediate (*n*=57) or high (*n*=14) risk according to the DRI, who were conditioned with BuCy (*n*=38) or BuFlu (*n*=33) followed by alloHCT from HLA identical sibling (*n*=23) or 9–10/10 matched unrelated donor (*n*=48). The stem cell were collected from peripheral blood (*n*=44) or bone marrow (*n*=27). GvHD prophylaxis consisted of calcineurin inhibitor combined with MTX plus ATG in alloHCT from unrelated donors. Engraftment was observed in all patients. The median time to neutrophil count (0.5 G/L) and platelet count (20 G/L) recovery was shorter after BuFlu in comparison with BuCy (18 days vs 22 days; *P*=0.045 and 13 days vs 20 days; *P*< 0.001), however peripheral blood stem cells were used more often after BuFlu regimen than after BuCy (88% vs 40%, p340/mm<sup>3</sup> on+21 day after transplant (2-year OS 77% vs 55%, *P*=0.034) and intermediate vs high DRI (2-year OS 78% vs 53%, *P*=0.068). In multivariate analysis higher AMC after alloHCT remained the only independent favorable prognostic factor for OS (RR 0.35 (95% CI 0.13–0.97), *P*=0.04). Our results suggest that early monocyte recovery after myeloablative Bu containing conditioning alloHCT is significant favorable predictor of outcome. In our experience both BuCy and BuFlu myeloablative regimens result in similar long-term survival after alloHCT in AML patients.

[P403]



Picture 1: Bilirubin, ALT levels and overall survival for BuCy and CyBu conditioning. Bilirubin and AST levels were significantly lower in the CyBu patient group. There was no difference in overall survival between the groups.

[P404]



**Disclosure of conflict of interest:** None.

**P405**  
**Effect of alemtuzumab dose de-escalation in reduced intensity conditioned unrelated donor haematopoietic stem cell transplantation for myeloid malignancy**

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The use of T-cell depletion as part of the conditioning protocol has the potential to improve the tolerability of allogeneic stem cell transplantation (HSCT) through the reduction in graft versus host disease (GVHD). Despite the wide spread adoption of this practice in many parts of the UK and Europe, definitive recommendations regarding the most appropriate dose remain elusive. Previous experience by our group with 100 mg of alemtuzumab combined with fludarabine and busulfan based conditioning demonstrated good long-term outcomes with low rates of GVHD. However, due to concerns of high relapse risk especially in patients with high-risk myelodysplastic syndrome and acute myeloid leukaemia, we instituted a policy change in 2013 to reduce the dose of alemtuzumab in the conditioning protocol from a total of

100–60 mg. We conducted a retrospective analysis of all consecutive patients undergoing reduced intensity unrelated allogeneic stem cell transplantation with fludarabine (150 mg/m<sup>2</sup>), busulfan (6.4 mg/kg IV or 12.8 mg/kg IV) and alemtuzumab (FB2C or FB4C, respectively) conditioning for neoplastic myeloid disorders between 2010 and 2016. Patients were subsequently analysed in two cohorts; those receiving 60 mg of alemtuzumab (n = 84) and those receiving 100 mg of alemtuzumab (n = 95). Apart from a decreased proportion of females in the 60 mg alemtuzumab group, the cohort was balanced across the different dose levels (Table 1). The long-term overall survival (OS) of the entire cohort was good with a 5 year OS of 51%. No significant differences in overall outcomes across the two groups were observed with a 5 year OS of 51% in the 60 mg group vs 49% in the 100 mg group (P = 0.39). Cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) was 42% and 21% and 39% and 24% in the 60 mg and 100 mg groups, respectively. Interestingly, age had a significant effect on NRM in the 100 mg (7% age < 50, 28% age 50–65 and 41% age > 65 P = 0.04), but not in the 60 mg group (11% age < 50, 19% age 50–65 and 24% age > 65 P = 0.8). The effect on relapse rate was not significant in either group (P = 0.6 and P = 0.11, respectively). This retrospective analysis did not demonstrate an overall improvement in transplant outcomes with dose de-escalation of alemtuzumab from 100 to 60 mg. In particular, we did not see the anticipated improvement in relapse rate in this cohort. Notably older patients seem to tolerate the 60 mg dose better due to the lower NRM. Prospective trials with accompanying translational work are required to determine the optimal dosing and schedule for this group of patients.

**Disclosure of conflict of interest:** None.

**P406**  
**High incidence of HHV-6 infection associated with bendamustine, cytarabine, etoposide and melphalan (BeEAM) conditioning regimen: Results of a monocentric and retrospective study**

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The disruption of BCNU (Carmustine) in France during several months led us to use Bendamustine in combination with

[P405]

**Table 1: Patient Demographic Data**

	60mg Campath (n=84)	100mg Campath (n=95)	p-value
<b>Age (median, range)</b>	60 (23-77)	59 (24-70)	=.2
<b>Gender, female %</b>	27% (23/84)	43% (41/95)	=.02
<b>HCT-CI</b>			=.39
0	23% (19/84)	31% (30/95)	
1	29% (24/84)	22% (21/95)	
>=2	48% (41/84)	46% (44/95)	
<b>Stem cell dose</b>			=.12
PBSC	100% (84/84)	97% (92/95)	
BM	0	3% (3/95)	
<b>Conditioning</b>			=.55
FB2	44% (37/84)	40% (37/95)	
FB4	56% (47/84)	60% (58/95)	
<b>Disease</b>			=.1
MDS RCMD	9% (8/84)	8% (8/95)	
MDS RAEB1/2	15% (13/84)	12% (11/95)*	
HypoMDS	4% (3/84)*	12% (11/95)*	
AML	65% (55/84)	56% (54/95)	
CML	2% (2/84)*	3% (3/95)	
MPN	0	5% (5/95)	
CMML	4% (3/84)	3% (3/95)	
<b>&lt;5% blasts</b>	100% (84/84)	99% (94/95)	
<b>HLA match</b>			=.93
Full	73% (61/84)	74% (70/95)	
9/10	27% (23/84)	25% (24/95)	
8/10	0	1% (1/95)	

Etoposide, Cytarabine and Melphalan (BeEAM) in a new high dose conditioning regimen before autologous transplant in relapsed/refractory (R/R) lymphoma patients (Visani *et al*, *Blood* 2011). We report our experience on the safety and efficacy of this regimen compared to the classical BEAM regimen. Ninety consecutive pts (BEAM = 60, BeEAM = 30) with R/R lymphoma were enrolled between December 2013 and September 2015 (BEAM from December 2013 to January 2015 and BeEAM from February to September 2015) in this retrospective study. Pts in complete or partial response after salvage therapy received high dose conditioning with Bendamustine (d-8 and d-7), Cytarabine (400 mg/m<sup>2</sup> continuous infusion from d-6 to d-3), Etoposide (200 mg/m<sup>2</sup> continuous infusion from d-6 to d-3) and Melphalan (200 mg/m<sup>2</sup> d-2) followed by ASCT on d0. Bendamustine was given at 200 mg/m<sup>2</sup>/d for the first 4 pts then 100 mg/m<sup>2</sup>/d for the 4 subsequent pts and finally at 120 mg/m<sup>2</sup>/d for the remaining pts (22 pts). Among the BEAM group, 68% had non-Hodgkin's lymphoma (NHL) and 32% Hodgkin's lymphoma (HL) compared to 87% and 13%, respectively, in the BeEAM group ( $P=0.014$ ). HHV-6 detection was performed by PCR for symptomatic pts (fever, rash or prolonged cytopenia). Patients were housed in single bedrooms with air filtration and received the same supportive care. Median age was 50 (18–66) and 56 (20–67) in the BEAM and BeEAM groups respectively and median of previous chemotherapy regimens was 2 (range: 1–5). Fifty two out of 90 patients were male (37/60 in the BEAM group and 15/30 in the BeEAM group). Pts were in CR (46.7% vs 56.7%) or PR (53.3% vs 43.3%) at time of transplant. There was no difference in terms of hematologic recovery (median = 11 days (range: 7–22)), blood and platelets transfusion, mucositis toxicity. There was no statistical difference in the incidence of acute renal failure when comparing the two groups. However, there was a very striking difference when considering the highest dose of Bendamustine when compared as well to the two others doses of Bendamustine ( $P < 0.00001$ ) as to the BEAM group ( $P=0.005$ ). Additionally, we also observed a high incidence of symptomatic HHV-6 infections (53.3% vs 8.3%,  $P < 0.00001$ ), digestive toxicity (36.6% vs 15%,  $P=0.03$ ) and a longer hospitalization duration (25 days (range: 18–59) vs 21 days (range: 18–32),  $P=0.001$ ) for patients in the BeEAM group overall. With a median follow up of 18.3 and 9.7 months for BEAM and BeEAM respectively, overall survival (93% vs 86%), transplant related mortality (0% vs 3%) and event free survival (83% vs 78%) were comparable. Overall, BeEAM regimen was associated with longer duration of hospitalization, higher rate of digestive toxicity and increased risk of symptomatic HHV-6 infection as compared to the BEAM regimen. In addition, higher doses of Bendamustine (200 mg/m<sup>2</sup>/d for two consecutive days) were associated with unacceptable high rate of acute renal toxicity. With a still short follow-up, the absence of benefit on disease control together with higher short term toxicity does not allow to recommend the use of BeAM instead of classical BEAM. Should it be used, we suggest that pts

should be carefully monitored for renal toxicity and for HHV-6 infection in case of symptoms.

**Disclosure of conflict of interest:** None.

**P407**

**High-dose treosulfan and melphalan for consolidation therapy in high-risk ewing sarcoma**

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Common toxicities observed after high dose chemotherapy with busulfan and melphalan for high risk Ewing sarcoma (ES) are generally well managed by current supportive care but some patients can develop severe complications. Treosulfan is an alkylating agent that has recently been used as a substitute of busulfan to prevent potential serious complications related to busulfan. Medical records of 7 ES patients undergoing autologous peripheral blood stem cell (aPBSC) transplantation after intravenous treosulfan (TREO) and melphalan (MEL) from 2011/6/1 to 2016/2/29 were analyzed with regard to toxicity and outcome. Patients were included into the study if they were eligible for the protocols activated in our institution for ES and presented reasons that did predict potential complications related to busulfan, such as previous radiotherapy on axial skeleton/pelvis or coexistence of high risk of epilepsy. As consolidation treatment patients received intravenous TREO 12 g/m<sup>2</sup> over 3 days and MEL 140 mg/sqm with support of aPBSC transplant and use of granulocyte colony stimulating factor. In those patients with lung metastases Total Lung Irradiation was performed at least 2 months after TREOMEL. Frequency of toxicity for TREOMEL was recorded with at least 6 months of follow-up and was evaluated according to NCI CTG common toxicity criteria. The median age at diagnosis of patients receiving TREOMEL was 16 years (range 13–25 years), 3 males and 4 females. 5 patients had localized disease at diagnosis with poor radiological or histological response to standard chemotherapy; one patient had lung metastases at diagnosis and one patient had relapsed disease with lung metastases. Before receiving TREOMEL the primitive tumour underwent radiation therapy in 5 cases (3 pelvis, 1 cervical vertebra, 1 sacrum), surgical resection in one case (tibia) and surgical resection plus radiation therapy in one case (fibula). 2 patients showed EEG abnormalities at high risk of developing epilepsy. The median number of infused CD34+ cells was  $5.9 \times 10^6$ /kg (range 3.4–15.9). Febrile neutropenia occurred in 5/7 patients and lasted one day in 3 patients and 2 days in 2 patients. Median time to granulocyte engraftment was 10 days (range 10–12 days); median time to platelet engraftment >20 000 was 10 days (range 9–16 days). Only one patient needed 2 red blood cells transfusions; 5 patients needed 1 platelet transfusion and 2 patients needed 2 platelet transfusions. None developed grade 3–4 stomatitis or grade 3–4

[P406]

Toxicity	BEAM (n=60)	BeEAM (n=30)	p
Acute renal failure	8, 3%	13, 3%	NS
• 200 mg/m <sup>2</sup>		75%	0.005 (vs BEAM)
• 120 mg/m <sup>2</sup>		4, 5%	
• 100 mg/m <sup>2</sup>		0%	
HHV-6 (PCR+)	8, 3%	53, 3%	$p < 0.00001$
Digestive toxicity	15%	36, 6%	$p < 0.03$
Hospitalization duration (days)	21	25	$p < 0.001$

**Main toxicities with Bendamustine containing conditioning regimen**

hematuria or grade 3–4 liver toxicity. Surprisingly, a patient became pregnant after 1 year and 10 months from transplantation. With a median follow-up of 24 months (range 8–65 months) 5 patients are alive in complete remission, one patient is alive with relapsed disease and one patient died for disease progression. These results, related to a limited cohort of patients, confirm the lower toxicity observed for treosulfan with respect to busulfan. Although more data are needed to clarify the role of treosulfan in ES, the impact of potential severe complications observed with busulfan, including infertility, should suggest its replacement with treosulfan in selected cases.

**Disclosure of conflict of interest:** None.

**P408**

**Immunoabsorption procedures prior to haploidentical allogeneic PBSCT could prevent graft failure in patients with hematological malignancies displaying anti-donor-specific HLA antibodies**

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Donor-specific anti-HLA antibodies (DSA) have been shown to be associated with a high risk of rejection in solid organ transplantation and with graft failure (GF) in allogeneic hematopoietic stem cell transplantation. A combination of anti-CD20 (Rituximab), plasma exchange (PE), and IVIg in 12 patients with additional buffy coat infusion in 5 among them prevented graft loss in all patients that became C1q negative before SCT. We addressed the question whether immunoabsorption in combination with Rituximab can also be applied in patients with DSA to prevent graft failure in haploidentical PBSCT. Four patients with acute myelocytic leukemia or myeloma in second complete remission were enrolled. The presence of DSA was determined by Luminex at pre BMT checking. Immunoabsorption was performed with polyclonal sheep anti-human IgG adsorbers (Miltenyi Biotec GmbH, Germany) on LIFE 18 apheresis system. In addition all patients received Rituximab 375 mg/kg bw in a single dose. Patients were conditioned with a reduced intensity regimen comprising TBI 2 Gy, Cyclophosphamide 29 mg/kg, and Fludarabin 150 mg/m<sup>2</sup>. All patients received Cyclophosphamide post BMT (PTCy) 100 mg/kg. Non-T-cell depleted PBSCT were transfused in a sequential manner in 2 doses each. The data of the patients and treatments is summarized in Table 1.

Two patients had a normal hematopoietic reconstitution and are alive at +21 and +15 months post-transplantation, one with hepatic GvHD; chimerism was 100% in peripheral blood on last follow up. One patient died following a graft failure. By a combination of Rituximab and repeated immunoabsorption prior to allogeneic PBSCT the titer of DSA could be lowered sufficiently to enable engraftment. IA turned out to be a safe procedure without relevant clinical side effects. Hematopoietic reconstitution was in the normal range in 2 of 3 evaluable patients.

**Disclosure of conflict of interest:** None.

**P409**

**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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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 35 children with beta thalassemia major who received allogeneic HSCT from HLA-matched unrelated donors with using a novel conditioning regimen. We retrospectively assessed 35 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 5 days, cyclophosphamide 120 mg/kg in 3 days, thiopeta 10 mg/kg in one day and 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. The median age of the patients was 6.9 years (range 14 month-15 year). Two of the patients were grouped in Class I and rest of them were Class II. The median serum ferritin level was 1.255 ng/ml (range, 585–5832). All of

[P408]

Patient No Diagnosis.	Donor specific antibody titer prior to IA	IA sessions	CD34 <sup>+</sup> cells x 10 <sup>6</sup> / kg bw	CD3 <sup>+</sup> cells x 10 <sup>7</sup> / kg bw	WBC > 1000/μl after SCT (days)	Platelet > 20,000 /μl after SCT (days)
1 AML	Anti B45 3300	3	3.24	34.20	17	27
2 AML	Anti-A32 – 2600 Anti-B8 - 5046	3	8.06	22.60	21	30
3 Myeloma	Anti-B58 2200	3	7.76	28.40	Graft failure	Graft Failure
4 AML	Anti-A24 22600	4	7.87	15.32	Ongoing	Ongoing

the donors were matched 10/10 with high-resolution HLA typing in GVHD direction but three of them 9/10 with graft failure direction. Twenty-three of them received BM (median TNC: 6.2X10<sup>8</sup>/kg) and 12 PBSC (median MNC:7.3x10<sup>8</sup>/kg) with median CD34+ cell number 7.20x10<sup>6</sup>/kg. The median neutrophil and platelet engraftment days were 13 and 14 days in PBSC and 17 and 20 days in BM group, respectively. Grade I-IV acute GVHD was observed in 7 patients (26%) and only one experienced limited chronic GVHD with only skin involvement. Mild to moderate VOD was seen in 13 patients (37%) and treated with defibrotide successfully. All patients except one are alive with full donor chimerism (between 95–100 %) with a median 12 months (range 3–49 months) follow-up. One patient died because of CMV pneumonia. 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 conflict of interest:** None.

#### P410

### Improved overall survival (OS) and reduced frequency of veno-occlusive disease (VOD) in acute myeloblastic leukemia (AML) patients after reduced-toxicity allogeneic stem cell transplantation (SCT)

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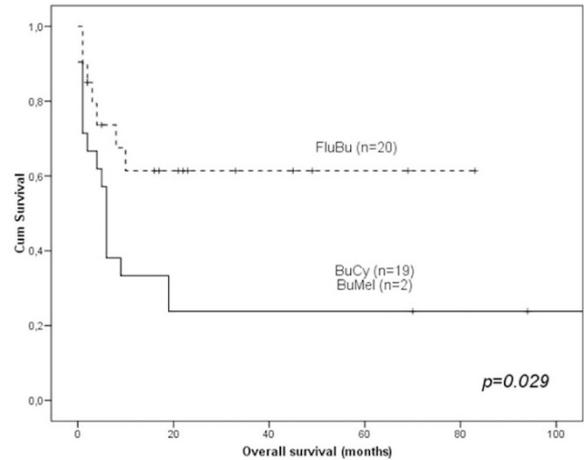
Allogeneic SCT is a potentially curative strategy for adult AML patients. Fludarabine-based conditioning regimens have been associated with better post-transplant OS, low transplant toxicity and reduced TRM. This work describes the experience of a single public SCT center in Montevideo (Uruguay). Since the beginning of our transplant program (1996), patients undergoing allogeneic SCT received mainly Busulfan-Cyclophosphamide (BuCy) conditioning regimen. Fludarabine-based regimens were increasingly incorporated from 2008. In this analysis, results of a retrospective comparison between standard- and reduced-toxicity regimens are shown. Retrospective, single center analysis of 41 AML patients undergoing allogeneic SCT between 1998 and 2016. Twenty one individuals received classical, standard toxicity regimens (GROUP 1): oral Busulfan (Bu) plus iv cyclophosphamide (BuCy)(n=19) and oral Bu plus iv Melphalan (BuMel) (n=2)(1998–2009). Twenty patients received reduced toxicity regimens (GROUP 2): iv Fludarabine plus oral Busulfan, from 8 to 16 mg/kg (2008–2016). Patient's characteristics are listed in Table 1. Data about cytogenetic risk of group 1 patients were available only in 9 individuals. Differences between groups were analyzed by T-student and Chi square tests. Survival was analyzed by Kaplan-Meier method and differences in survival between groups were evaluated by log rank test. No differences were found between groups regarding gender, SC source, disease status at SCT, type of donor and number of CD34+ cells infused. Patients in group 2 were significantly older (median age for groups 1 and 2: 32 vs 38, P=0.021). GVHD prophylaxis protocols included ATG in a higher frequency in group 2. No differences between groups 1 and 2 were observed in neutrophils recovery (median days to ANC > 500/μL: 9 vs 8 respectively, P=0.78) and platelets recovery (median days to platelets > 20 000/μL: 8 vs 5 respectively, P=0.51). Patients in group 1 required more red cell transfusions (median packed RBC: 3 vs 1, P=0.048). No differences were observed regarding platelets transfusion requirements or length of hospitalization. Post-SCT OS was significantly better in group 2 (3 years-OS group 1: 23%; group 2: 61%; P=0.029) (figure 1). There were no significant differences between groups regarding frequency of mucositis, diffuse alveolar haemorrhage, sepsis, acute and chronic GVHD. VOD was more frequent in group 1 (4/21 vs 0/20, P=0.03). TRM mortality was higher in group 1 (6/21 vs 3/20), being this difference not statistically significant (P=0.29). As it was reported by others, the use of Fludarabine-based conditioning regimen was associated

with a significantly better post-SCT OS and a reduced frequency of VOD in AML patients. Reduction in TRM and differences in the frequency of described complications are not statistically significant probably due to the small size of this sample.

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[P410]



**Disclosure of conflict of interest:** None.

#### P411

### Low toxicity profile of the combination of carmustine, etoposide, cytarabine, and cyclophosphamide (BEAC) as an alternative conditioning regimen in autologous haematopoietic cell transplantation for lymphoma patients: a single center's experience

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Since March 2016, given the limited availability of Melphalan, we administer the BEAC regimen (Carmustine, Etoposide, Cytarabine, and Cyclophosphamide), instead of the gold standard conditioning regimen BEAM, followed by autologous haematopoietic cell transplantation (AHCT) in relapsed or refractory Hodgkin (HL) and Non-Hodgkin (NHL) lymphoma patients. The primary goal of this analysis was to assess the immediate related toxicity of this alternative regimen. We used BEAC (Carmustine 300 mg/m<sup>2</sup>, Etoposide 800 mg/m<sup>2</sup>, Cytarabine 800 mg/m<sup>2</sup>, and Cyclophosphamide 140 mg/kg) in 22 consecutive lymphoma patients (13 HL, 9 NHL) who underwent AHCT for relapsed or refractory disease. The median age of the patients was 42.5 years (17–64). They all received peripheral stem cell grafts with a median CD34+ cell dose of 5.13 × 10<sup>6</sup>/kg cells (2.07–15.78). Disease status post salvage treatment (at AHCT) was complete remission (CR) in 6, partial remission (PR) in 11 and progressive disease in 5. The disease was chemosensitive to salvage therapy in 17/21 patients. Median follow up was 112 days (31–224). Toxicity was assessed according to the WHO toxicity scale grading. All patients engrafted successfully. Median time for engraftment was day +10 (d+10) for neutrophils (> 500/mm<sup>3</sup>) and d+11 for platelets (> 20 000/mm<sup>3</sup>, without transfusion within the previous 3 days). Patients were hospitalized for a median of 19 days (16–28). No treatment-related mortality occurred. Two patients died due to disease progression (both NHL patients, on d+143 and d+63). Toxicity assessment until d+30 is presented in Table 1:

**Table 1: Toxicity grading until d+30**

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Oral mucositis, n	10	11	1	none	none
Vomiting, n	9	2	8	3	none
Diarrhea, n	9	2	7	4	none
Transaminases, n	18	2	1	1	none
Bilirubin, n	22	none	none	none	none
Creatinine, n	22	none	none	none	none

Moreover, no hemorrhagic cystitis or macroscopic hematuria, and no cardiac events were encountered. Febrile neutropenia was recorded in 4 and bacteremia in 9 patients (7 gram+, 2 gram-, 2/9 related to central venous catheter), with fever  $\leq$  grade 2 in all cases. During d+30-100 two patients presented fever of unknown origin, and 3 patients had upper or lower respiratory infections, with no other adverse events being recorded. In terms of disease best response within 3 months post AHCT (18/22 patients evaluated), 11 patients achieved or sustained CR, 5 PR (1 of these patients eventually died due to disease progression), 1 relapsed and 1 succumbed due to disease progression (no response). According to our preliminary results, the early toxicity profile of BEAC is very low, the regimen is easily tolerable for the patients, and without any treatment-related mortality. Its use as an alternative conditioning regimen in AHCT for lymphoma patients seems feasible. Further investigation including more patients and comparative analysis to other conditioning regimens are warranted for reliable conclusions on the toxicity and efficacy of BEAC. **Disclosure of conflict of interest:** None.

**P412****Maximal concentration of intravenous busulfan is a determinant of veino-occlusive disease in bone marrow transplanted children**

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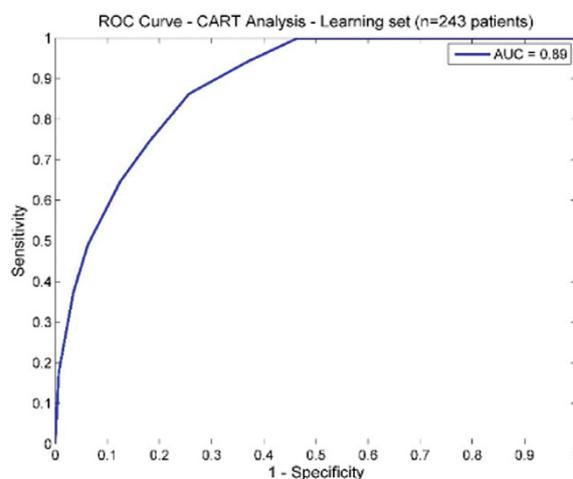
Veino-occlusive disease (VOD) is a potentially fatal adverse event caused by intravenous (IV) busulfan used in bone marrow transplantation (BMT) conditioning. The objective of this study was to identify determinants of VOD in children treated by IV busulfan. This was a retrospective analysis of data collected in 293 children from two BMT centers over 10 years. VOD was diagnosed according to modified Seattle criteria. Individual pharmacokinetic data, including busulfan area under the concentration-time curve (AUC) and maximal concentration (C<sub>max</sub>) were estimated in all children by using a validated Bayesian approach.<sup>1</sup> We examined the relationships between the occurrence of VOD and available data in a learning ( $n=243$  patients) and validation set ( $n=50$  patients) obtained by random splitting. Logistic regression was used as a continuous statistical model. In addition, we used classification and regression tree (CART) analysis, a machine learning and binary partitioning technique, to identify determinants of VOD and their optimal cut-off values. The predictive performance of variables within both models was assessed by

statistical tests and receiver operating characteristics (ROC) curve analysis. 178 children were treated at the Institute of Pediatric Hematology and Oncology of Lyon and 115 at the Children's Hospital of Los Angeles. Most of them received busulfan as a fourth daily administration over 4 days (96%), only 11 children received busulfan once daily. Median age and body weight (min-max) were 4 years old (0.2–21) and 17.8 kg (3.2–137), respectively. Median cumulative AUC and C<sub>max</sub> (min-max) were 69.8 mg h/L (16.3–100.6) and 1350 ng/ml (590–3830), respectively. The overall incidence of VOD was 26.2%. In the learning sample, the final logistic regression model included two variables associated with VOD: an ideal body weight (IBW)  $<$  9kg (OR=2.30) and busulfan C<sub>max</sub> over the entire conditioning (OR=3.25). This model had a ROC curve AUC of 0.618, a positive predicted value (PPV) and a negative PV (NPV) of 90% and 77.3% respectively. In the validation set, this model showed similar results: ROC AUC of 0.612, a PPV of 100% and a NPV 77.1%. The CART analysis identified 12 binary variables predictive of VOD in the learning set. The three strongest predictors of VOD were C<sub>max</sub>  $\geq$  2000 ng/ml on the first day of therapy (OR=30), height  $<$  75.5 cm (OR=3.1), and use of cyclophosphamide in the conditioning (OR=10.5). The AUC of the ROC curve associated with this predictive tree was 0.89 in the learning set and 0.63 in the validation set. Maximal concentration of IV busulfan is a significant predictor of VOD in BMT children. Those results support the need for busulfan therapeutic drug monitoring and target-oriented dosing approach.<sup>2</sup>

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**Disclosure of conflict of interest:** None.

**P413**

**Mitoxantrone, etoposide, cytarabine, and melphalan (NEAM) followed by autologous stem cell transplantation (auto-SCT) as alternative conditioning regimen in Hodgkin's and non-Hodgkin's lymphomas: experience in latin American center**

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High dose chemotherapy followed by autologous stem cell transplantation (auto-SCT) is the worldwide treatment used in chemosensitive Hodgkin and non-Hodgkin lymphomas. Carmustine, Etoposide, Cytarabine and Melphalan (BEAM) is one of the most used conditioning regimens. Difficulties in obtaining Carmustine in our region have led to search for alternatives to BEAM. We report the clinical outcome of 9 patients who received an alternative conditioning regimen based on Mitoxantrone, Etoposide, Cytarabine and Melphalan (NEAM) between September 2015 and September 2016 in a Public Center in Montevideo, Uruguay, a Latin American country of 3.4 million population. These results are compared with historical data from our Service using BEAM as conditioning followed by auto-SCT in lymphoma patients. Nine patients were enrolled to receive NEAM: Mitoxantrone 6 mg/m<sup>2</sup> day -6 to -4, Etoposide 100 mg/m<sup>2</sup> every 12 hours and Cytarabine 100 mg/m<sup>2</sup> every 12 hours day -6 to -3, and Melphalan 140 mg/m<sup>2</sup> day -2, followed by auto-SCT. The median age was 51 years (19–63); five non-Hodgkin lymphomas (NHL) and four Hodgkin lymphomas (LH). Six patients were in partial remission (PR), two in complete remission (CR), and one with progressive disease at time of auto-SCT. NEAM patients were compared with a historical control group of patients receiving BEAM regimen (n=167). Differences between groups were analyzed by T-student and  $\chi^2$ -tests. Median CD 34+ cells infused in NEAM and BEAM groups was 3.48 × 10<sup>9</sup>/kg (3.09–8.72) and 4.14 × 10<sup>9</sup>/kg (1.02–25.6), respectively (P=0.42). The median time to neutrophil recovery (> 500/ $\mu$ L) was 16 days (11–22) and 10 days (3–30) (P=0.000017) and median time for platelets recovery (> 20 000/ $\mu$ L) was 15 days (9–30) and 8 days (1–49) (P=0.018) respectively, for NEAM and BEAM patients. Median duration of hospitalization was 34 days (24–55) with NEAM and 27 days (15–59) with BEAM (P= 0.002). Among NEAM patients, 88% had one or more febrile episodes during neutropenia. No case of grade III or IV mucositis was described. There was no transplant-related mortality (TRM: 0%) associated with the use of NEAM regimen. At the present, all NEAM patients are alive, two of them in relapse (22%). Due the difficulties in obtaining Carmustine in our region, NEAM can be considered as a feasible alternative to BEAM. However, despite the sample was small enough to draw conclusions, we find that NEAM presents prolonged aplasia of significant value, we are currently exploring conditioning regimens followed by auto-SCT in Hodgkin's and Non-Hodgkin's lymphomas based on bendamustine, etoposide, cytarabine and melphalan.

**Disclosure of conflict of interest:** None.

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**More frequently employed conditioning regimens in allo-HSCT: conventional or reduced intensity but myeloablative**

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At present, decision-making about conditioning regimens for allogeneic HSCT is based on patient's and donor's features, and disease characteristics. During the last years, terms as

'myeloablative/non-myeloablative/reduced-intensity' have been frequently employed in a confusing and unequal way among the different centers. Knowing the expected intensity and myeloablative effect from each regimen is very useful and constitutes the aim of this analysis. We have analysed the severe neutropenia (ANC < 500 per mL), and thrombocytopenia durations (platelets < 20 000 per mL), the need for platelet concentrates transfusion and the duration of the inpatient period of the 223 allo-HSCT carried out during the last four years in our centre. These data are reported according to the conditioning regimen used and to the type of transplant performed. Then, they are compared among them in order to establish intensity ranks.

**RESULTS:** population characteristics are described in Table 1: [P414]

N	223
Gender (male/female)	126 (56.5%)/97 (43.5%)
Age [median] (years)	53 (8 – 72)
Diseases	AML 99, ALL 28, Lymphoma 25, MDS 23, cMPD 20, MM 9, BMF 8, CLL 7, Other 4
Source of progenitors (PB/BM)	200/23
Donor	Unrelated 123 Family 100 (25 haplo-identical)
Conditioning regimen	Busulphan-based 155 Melphalan-based 23 TBI-based 22 Others 23

Conventional intensive regimens (Bu-Cy2, Cy-Bu2, TBI-Cy) reported more days of severe neutropenia and greater need of platelet concentrates transfusion. The regimens with less days of severe neutropenia, less need of platelet concentrates transfusion and fewer days of admission were Flu-Bu3 and Flu-Bu2. Allotransplants carried out with stem cells from BM presented more days of severe neutropenia and longer hospital stay. Similar platelet transfusion need was reported. Haplo-identical allotransplants reported more days of severe thrombocytopenia, but were not associated to longer neutropenia or longer hospital stay than the others. These data are described in detail in Table 2.

	ANC <500/mcL (days)	Platelets <20000/mcL (days)	PC transfused (units)	Hospital stay (days)
Family and URD PBST				
HSCT (N=178)				
Bu-Cy2, Cy-Bu2, TBI-Cy (N=25)	17 (8-27)	7 (1-14)	6 (1-24)	32 (24-80)
Flu-Mel140 (N=22)	16.5 (9-26)	8 (0-17)	5.5 (1-15)	34 (25-53)
Flu-Bu4 (N=41)	14 (7-34)	4 (0-21)	3 (0-13)	32 (26-87)
Flu-Bu3 (N=17)	13 (7-16)	5 (0-8)	2 (0-5)	29 (25-53)
Flu-Bu2 (N=46)	13 (3-41)	3 (0-36)	2 (0-23)	30 (24-59)
Family and URD BM HSCT (N=20)	17 (10-27)	6 (0-19)	5 (0-25)	34 (28-63)
Haplo-identical PBSC HSCT (N=25)	14 (9-23)	11 (2-26)	7 (1-23)	33 (18-123)

Flu-Bu: the number (4, 3 or 2) expresses the doses of busulphan administered at 3.2 mg/kg/day. PC: Platelet concentrates Data is expressed in medians. Within the analysed conditioning regimens, an intensity rank is established regarding the myelosuppression induced (in descending order): conventional intensive regimens (Cy-Bu2, Bu-Cy2, TBI-Cy), Flu-Mel, Flu-Bu4, Flu-Bu3 and Flu-Bu2. All of them induced severe neutropenia and thrombocytopenia, and for that reason they must be considered myeloablative regimens.

**Disclosure of conflict of interest:** None.

**P415**

**Myeloablative allogeneic stem cell transplant for AML and MDS: the impact of advanced age in the outcome**

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Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative option in high risk myeloid hematological malignancies. Myeloablative conditioning (MAC) regimen has been proven to be effective in the control of high risk diseases in advanced age patients. **OBJECTIVE:** The aim of this study was to analyze the efficacy of myeloablative Allo-HSCT in two cohorts of patients considering their age at transplant. We also analyzed the incidence of acute and chronic Graft versus Host Disease (GvHD) and procedure related outcomes [Overall Survival (OS), Progression Free Survival (PFS) and Transplant

Related Mortality (TRM)]. Between 2005 and 2013, 134 patients [92 (68.7%) AML and 42 (31.3%) MDS] who underwent to myeloablative Allo-HSCT were retrospectively analyzed. The median age was 49 years (IQR 36–57). Both groups were divided regarding their age at Allo-HSCT [group 1, age ≥ 55 years (n=41) and group 2, age < 55 years (n=93)]. Patient's characteristics are shown in Picture 1. Data were collected as either continuous data and compared by two-tailed unpaired t-test or Mann-Whitney test, or as categorical variables and compared by Chi-square. The procedure related outcomes were analyzed with the Kaplan-Meier test. The incidence of acute GvHD grade II–IV was similar in both groups (43.9% in group 1 and 42.2% in group 2, P=0.761). The mean day to acute GvHD (grade II–IV) development was 38 days in group 1 and 40 days in group 2. The most involved organs in both groups were skin (group 1: 94.4% and group 2: 72.1% [P=0.258]) and gut (group 1: 55.6% and group 2: 62.8% [P=0.598]). At day +100 post-transplant 123 patients were alive and evaluable for chronic GvHD. The incidence of cGvHD development was similar between group 1 and 2 (61.1% versus 68.4%, respectively, P=0.140). However, severe grade

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Patients characteristics*	(group 1) Age ≥ 55 years (n=41)	(group 2) Age < 55 years (n=93)	p-value
Median follow up, months	32	50	0.268
Median age, years (IQR)	61 (58-64)	42 (32-50)	---
Median donor age, years (IQR)	51 (32-60)	36 (29-47)	---
Gender, male (%)	20 (48.8)	49 (52.7)	0.677
1st CR at transplant (%)	27 (65.9)	53 (57.0)	0.453
Disease, AML (%)	26 (63.4)	66 (71.0)	0.385
Source, bone marrow (%)	40 (97.6)	75 (80.6)	0.007
<b>EBMT Score (%)</b>			<b>0.529</b>
0-2	12 (29.3)	36 (38.7)	
3-4	23 (56.1)	43 (46.2)	
>=5	6 (14.6)	14 (15.1)	
<b>HCT-CI Sorrow (%)</b>			<b>0.982</b>
0	8 (19.5%)	17 (20.5%)	
1-2	15 (36.6%)	31 (37.3%)	
>=3	18 (43.9%)	35 (42.2%)	
<b>Donor (%)</b>			<b>0.267</b>
Matched related	23 (56.1)	38 (40.9)	
Matched unrelated	9 (22.0)	35 (37.6)	
Mismatched donor	9 (22.0)	20 (21.5)	
<b>Conditioning regimen (%)</b>			<b>0.005</b>
FluBu4	36 (87.8)	57 (61.3)	
BUCY and TBI-CY	4 (9.8)	32 (34.4)	
<b>GvHD prophylaxis, CsA-mycophenolate (%)</b>			<b>0.004</b>
ATG use (%)	0 (0.0)	16 (17.2)	
<b>Main cause of death (%)</b>			<b>0.806/0.135</b>
GvHD /infection	7 (29.2)/7 (29.2)	10 (32.3)/4 (12.9)	
<b>Acute GVHD</b>			
Grade II-IV (%)	18 (43.9)	43 (42.2)	0.761
Grade III-IV (%)	5 (12.1)	16 (17.2)	0.819
Mean time to acute II-IV GVHD, days	38	40	0.496
<b>Chronic GVHD development</b>			<b>0.445</b>
Grade of GVHD (%)			
Mild/Moderate/Severe	3 (8.3%)/9 (25.0%)/9 (25.0%)	18 (23.7%)/27 (35.5%)/7 (18.4%)	0.140

of cGvHD was high in group 1 patients (25.0% versus 18.4%). With a median follow up of 43 months (IQR, 9–70) the probability of OS was significantly low ( $P=0.004$ ) in group 1 (46.3%  $\pm$  7.8) compared with group 2 (67.0%  $\pm$  4.9). PFS was also significantly low ( $P=0.003$ ) in group 1 (41.5%  $\pm$  7.7) compared with group 2 (66.3%  $\pm$  4.9). TRM at 43 months was higher in group 1 compared with group 2 (34.1% versus 17.2%). Mortality due to relapses was also higher in group 1 (17.0% versus 12.9%). Most of the patients died during the first 24 month. Comparing both groups at this time (24 months post-transplant), TRM was higher in group 1 compared with group 2 (26.8% versus 14.0%). Deaths due to relapse were also higher in this group (17.1% versus 10.7%). In our series, myeloablative conditioning regimen provides good survival rates and disease control in high risk hematopoietic diseases, however in patients aged  $\geq 55$  years confers high toxicity. It may be necessary to evaluate other strategies in this group of patients.

**Disclosure of conflict of interest:** None.

#### P416

##### **Radiation-free cytoreductive regimens and T-cell depleted allogeneic stem cell transplants (HSCT) for the treatment of 65 pediatric patients with hematologic malignancies**

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One of the missions of our Service in the treatment of pediatric patients with hematologic malignancies has been to minimize the risks of graft-versus-host disease (GvHD) and decrease the late effects of transplantation. We developed two radiation-free cytoreductive regimens for HSCT for the treatment of children with hematologic malignancies: (1) Busulfan (Bu) 0.8–1.0 mg/Kg/dose  $\times$  10–12 doses, Melphalan (Mel) 70 mg/m<sup>2</sup>  $\times$  2 doses, and Fludarabine (Flu) 25 mg/m<sup>2</sup>/dose  $\times$  5 doses primarily for myeloid malignancies, and (2) Clofarabine (Clo) 20–30 mg/m<sup>2</sup>/dose  $\times$  5, Thiotepa (10 mg/Kg/dose) and Mel (70 mg/m<sup>2</sup>/dose  $\times$  2) for lymphoid or myeloid malignancies. Grafts included G-CSF mobilized peripheral blood stem cell grafts (PBSC) that were T-cell depleted by Isolex positive CD34 selection and sheep-RBC rosetting or by CliniMACS positive CD34 selection, or soybean agglutinin E-rosette depleted bone marrow (BM) grafts. All patients received Rabbit ATG (Thymoglobulin) for the prevention of rejection, and G-CSF for the promotion of engraftment. Forty-four patients received cytoreduction with Bu Mel Flu on the Pediatric BMT Service from August 2001 to December 2015, aged 1.9–29.2 years (median 13.8 years), including 28 patients transplanted < 2010 and 16 pts  $\geq$  2010 (Table 1). Patients older than 20 years had secondary leukemia following a prior childhood malignancy. Diagnosis included: Acute myelogenous leukemia (AML)  $N=29$ , myelodysplastic syndrome (MDS)  $N=13$ , and acute lymphoblastic leukemia  $N=2$ . Thirteen patients had secondary MDS/AML. Donors were HLA-matched related ( $N=11$ ), HLA-mismatched related ( $N=4$ ), matched unrelated ( $N=12$ ) or mismatched unrelated ( $N=17$ ). Twenty one patients received cytoreduction with Clo Mel Thio from March

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2010 to November 2015, aged 0.6–32 years (median 14.3 years) (Table 1). Patients older than 20 years had secondary leukemia following a prior childhood malignancy. Diagnosis included: ALL  $N=10$ , AML  $N=9$  and MDS  $N=2$ . Four patients had secondary MDS/AML. Donors were HLA-matched related ( $N=9$ ), HLA-mismatched related ( $N=2$ ) or unrelated ( $N=10$ ). The respective two year overall survival (OS) and disease-free survival (DFS) were as follows: Bu Mel Flu 2000–2009 cohort: 74% and 45%, Bu Mel Flu 2010–2015 cohort: 82% and 70%, Clo Mel Thio cohort: 71% and 58%. For the Bu Mel Flu cohorts, five patients developed grade 2–4 GvHD. Sixteen patients died. Cause of death was GvHD ( $N=4$ ), transplant related mortality (TRM) ( $N=2$ ) and relapse ( $N=10$ ); seven of the 10 relapsed patients had advanced disease ( $\geq$ CR2). For the Clo Mel Thio cohort, only one patient developed grade 2–4 GvHD. Seven of the 21 patients died. Cause of death was TRM  $N=2$  and relapse ( $N=5$ ); all but one of the relapsed patients had advanced disease. In summary, the Bu Mel Flu and Clo Mel Thio cytoreductive regimens followed by T-cell depleted transplants were overall well tolerated, led to promising transplant outcomes without the use of irradiation and with minimal GvHD.

**Disclosure of conflict of interest:** None.

#### P417

##### **Reduced intensity conditioning combined with <sup>188</sup>Rhenium anti-CD66 radioimmunotherapy for allogeneic hematopoietic cell transplantation in patients with relapsed multiple myeloma**

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Allogeneic hematopoietic cell transplantation (HCT) is reserved for a group of high risk multiple myeloma (MM) patients having relapsed after high-dose melphalan and autologous transplantation. In general, reduced-intensity conditioning (RIC) regimen are applied in this patient group with the aim of reducing transplant related mortality (TRM). However, relapse of disease remains a major challenge after allogeneic HCT. To address this issue, we added radioimmunotherapy (RIT) to a conventional RIC regimen. We have used a <sup>188</sup>Rhenium Anti CD66 antibody in combination with a RIC conditioning regimen. This  $\beta$ -emitter leads to a so called 'cross-fire' effect allowing for bone marrow doses of 20 Gy and in parallel may target CD66 on myeloma cells. We hypothesized that this strategy may decrease the incidence of relapse. So far, we have treated nine patients with high risk relapsed multiple myeloma. All patients had received one ( $n=7$ ), two ( $n=1$ ) or three ( $n=1$ ) high-dose regimens and autologous HCT. Conditioning therapy was Flu/Mel ( $n=5$ ), Flu/Bu ( $n=2$ ) or Flu/Treo ( $n=1$ ). Flu/Cy and 2 Gy TBI were applied before haploidentical transplantation. Patients received G-CSF mobilized PBSC from unrelated ( $n=8$ ) or haploidentical ( $n=1$ )

Regimen	N	Age median range (years)	Diagnosis	Stage CR1	Stage $\geq$ CR2	Secondary MDS/AML	Median F/U (months)	2 year OS/DFS
Bu Mel Flu	44	13.8 1.9-29.2	ALL N=2 AML N=29 MDS N=13	28	16	13	40	2000-2009: 74% / 45% 2010-2015 82% / 70%
Clo Mel Thio	21	14.2 (0.6-32.0)	ALL N=10 AML N=9 MDS N=2	8	13	4	29	71% / 51%

donors. Either Tacrolimus/ Methotrexate/ Bortezomib or Cyclosporine A/ Methotrexate were used for GvHD prophylaxis. Early extramedullary toxicity was limited. Neutrophil and platelet engraftment was timely and complete in time in seven of nine cases. All patients achieved full donor chimerism around day fifteen after HCT. Severe acute graft-versus-host-disease (GvHD grade III-IV) occurred in two patients and was lethal in both cases. Two patients have experienced extramedullary relapse, one of them in the central nervous system and the other in the soft tissue. In two patients, a transplantation-associated thrombotic microangiopathy (TA-TMA) was diagnosed. Four patients are alive and in complete remission. We conclude that the combination of a RIC regimen with a <sup>188</sup>Rhenium anti-CD66 radioimmunotherapy is safe and feasible. The incidence of GvHD, TA-TMA and extramedullary relapse will be monitored closely and will be presented in a larger patient cohort.

**Disclosure of conflict of interest:** None.

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##### Reduced toxicity conditioning tiotepa-fludarabine-busulfan (TBF) vs fludarabine-busulfan (BF) in HSCT with tacrolimus/sirolimus as GvHD prophylaxis

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The sirolimus/tacrolimus (sir/tac) combination has been associated with a better outcome after allogeneic hematopoietic stem cell transplantation (allo-HSCT) when compared with conventional prophylaxis for graft vs host disease (GvHD) as cyclosporine/methotrexate in the true reduced-intensity conditioning (RIC) setting but not in the myeloablative setting. In moderate-intensity regimens as thiotepa/busulphan/fludarabine (TBF), the sir/tac combination has not been evaluated. From January 2009 to December 2015, all consecutive RIC-allo-HSCT recipients who received sir/tac combination to prevent GvHD in three Spanish institutions were included in the study. The reduced-toxicity regimens used in this study where: (A) intravenous busulphan (6.4 mg/kg) and fludarabine 150 mg/m<sup>2</sup> (BF), or (B) thiotepa days -4 and -3 and 10 mg/kg if > 55 yrs old or 5 mg/kg if < 55 yrs old on days -6 and -5 added to the BF regimen (TBF). The GVHD prophylaxis with sir/tac was given as detailed elsewhere (Cutler C *Blood* 2014) and was consistent within the 3 center. The outcomes of the procedure according to the conditioning regimen were analyzed. Overall, 125 patients were included: 66 TBF and 59 patients in the BF group, with a median follow-up of 22 months (range 3–83) and no difference in the median age (56 vs 58 years old). There were more males (71% vs 47%,  $P=0.007$ ) and more female donors to male recipients (35% vs 13%,  $P=0.006$ ) and more patients with lymphoid diseases and previous ASCT in the TBF group (27% vs 12%,  $P=0.03$ ), whereas there were more unrelated donors in the BF group (56% vs 76%,  $P=0.02$ ). Other baseline characteristics were balanced between the 2 groups (Table 1). Sir/tac prophylaxis had to be discontinued in 48% and 65% patients in the TBF and BF groups, respectively. Toxicity was the main reason for discontinuation in the TBF group. The most frequent toxicities were renal injury (TBF 30% and BF 10%) and neurologic impairments (TBF 6%, BF 5%). In the BF group, the main reason of discontinuation was relapse or a mixed chimera. Patients who received TBF presented higher incidence of extensive chronic GvHD (65% vs 39%,  $P=0.02$ ), higher NRM at 100 days (21% vs 4%) and at 2 years (42% vs 13%,  $P=0.001$ ). there were no differences in OS (2

years) between both groups ( $49 \pm 6.9\%$  vs  $80 \pm 5.5\%$ ,  $P=0.001$ ) (Figure). There were no differences regarding to acute GvHD 2–4 (39% vs 36%,  $P=0.31$ ), acute GvHD 3–4 (23% vs 13%,  $P=0.08$ ), or relapse (up to 2 years, 22% vs 14%,  $P=0.3$ ) between the 2 groups, either. The combination of sir/tac as GvHD prophylaxis was associated with higher incidence of chronic GVHD and NRM in patients receiving conditioning regimen with TBF compared to those receiving BF. There were no differences in OS between both groups.

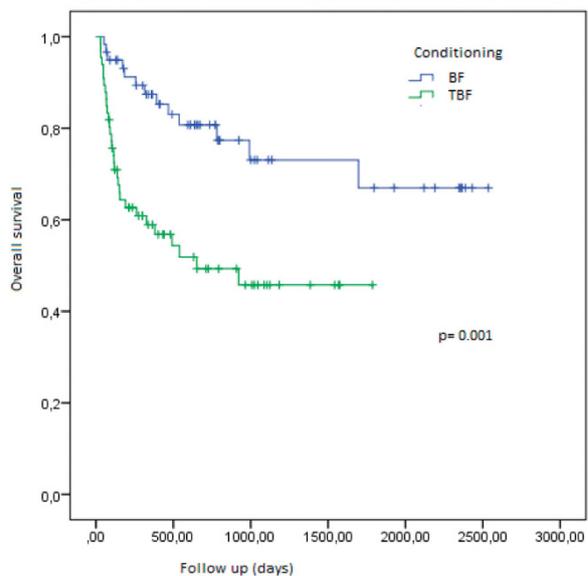
[P418]

Table 1: Characteristics based on the conditioning regimens

Characteristics	TBF (n=66) N (%)	BF(n=59) N(%)	p
Hematological malignancy			
Myeloid	38 (57)	54 (91)	<0.001
Lymphoid	23 (35)	1 (2)	
Others	5 (8)	4 (7)	
State of disease before HSCT	48 (73)	38 (65)	0.44
CR	10 (15)	9 (15)	
PR	8 (12)	12 (20)	
NR/Progression			
Match	55 (83)	50 (84)	0.8
Mismatch	11 (17)	9 (16)	
ATG	11 (17)	8(14)	0.55
HCT-CI <3	33 (50)	39 (66)	0.69
≥3	33 (50)	20 (34)	

CR complete remission, PR partial remission, NR Non remission, HCT-CI Hematopoietic Cell transplant comorbidity index.

Figura 1: Overall survival between patients with TBF vs. BF conditioning



**Disclosure of conflict of interest:** None.

#### P419

##### Reduced-intensity conditioning regimen with fractionated total body irradiation of 6 Gy and cyclophosphamide 60 mg/kg for allogeneic hematopoietic stem cell transplant is well tolerated and offers a potential disease control as treatment of acute leukemia and lymphoproliferative disorders

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The use of reduced-intensity conditioning regimens (RIC) before allo-HSCT is widely extended since it preserves the

graft-versus-leukemia effect but reduces treatment related mortality. However, there exist different RIC regimens with diverse outcomes and the choice of the RIC regimen relies on the type of disease treated, experience of the center and previous therapies. This is a retrospective study of patients treated in our institution within 01/2000 and 12/2015. The RIC regimen consisted of fractionated total body irradiation (FTBI) of 6 Gy administered in 3 consecutive days (2 Gy/day) and cyclophosphamide 60 mg/kg given in 2 days (30 mg/kg/day). Post-transplant immunosuppression consisted of CsA started the day before allo-HSCT and short MTX on days 1, 3 and 6 after transplantation. For patients receiving transplant from unrelated donors, anti-thymocyte globulin at a dose of 5 mg/kg (2.5 mg/kg/day for 2 days at day -2 and -1) was used as part of the immunosuppressant therapy. 78 patients (median age: 54 years; range: 36–64 years) were included. The median HCT-CI was 0.5 (range: 0–4). Primary disease was multiple myeloma (MM) in 45 (58%), AL/MDS in 14 (18%), CLL in 10 (13%), NHL in 9 cases (12%). 51 patients (65%) received transplant from matched related donors, 22 (28%) from matched unrelated donors and 5 (6%) from mismatched unrelated donors. Female to male mismatch incidence was 23% ( $n=18$ ). Most of the patients ( $n=77$ ) received a peripheral blood graft. 1 patient received a second allogeneic transplant. MM patients were transplanted in a “tandem” autologous-allogeneic HSCT program in 42 cases. The median number of chemotherapy lines prior to transplant was 3.5 in CLL, 2.8 in MM and 2.5 in NHL. 62 patients (91%) engrafted by day 28 post transplant. Neutrophil engraftment occurred at a median of 19 days (range: 14–35 days) and platelet engraftment at a median of 18.5 days (range: 9–103 days). Full donor chimerism was observed in 62 out of 67 patients (92%) having survived by day 180. Primary graft rejection was observed in 4 patients. Treatment related toxicities consisted of grade 3/4 mucositis in 53 patients (68%), grade 3 (range: 2–4) cardiac toxicity in 6 patients (8%), grade 3 (range: 3–4) hemorrhagic complications in 4 patients (6%) including 3 cases of hemorrhagic cystitis and secondary malignancies in 4 patients, this within a median follow-up of 6.6 years. Infectious complications during aplasia included fever of unknown origin ( $n=52$ ), bacteremia ( $n=17$ ) with 3 cases of bacteremia with severe sepsis and 8 cases of infections defined by bacterial foci. Incidence of aGvHD was 33% with 3 cases of grade 3/4 refractory aGvHD. cGvHD occurred in 30 pts (39%). The non-relapse mortality (NRM) at 100 days was 5% including 2 cases of septic shock, 1 case of acute cardiac toxicity and 1 case of aGvHD. The NRM at 1 year was 10%. 1-year survival rates were 60% in AL, 80% in CLL and 88% in NHL with extended survival benefit. In AL patients, the relapse incidence was 36% comprising 2 patients who progressed during conditioning. The 1-year survival rate in MM patients was 77%. In MM patients who were in complete response prior to transplant, median overall survival was 4.6 years. The used RIC regimen resulted in durable donor engraftment with an acceptable toxicity profile permitting efficient disease control in the described cohort.

**Disclosure of conflict of interest:** None.

#### P420

##### **TBI-based myeloablative conditioning improves engraftment for $\alpha\beta$ -T cell depleted haploidentical HSCT in pediatric malignancies**

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Graft manipulation using selective depletion of  $\alpha\beta$ -T cells provides a source for haploidentical hematopoietic stem cell transplantation (haplo-HSCT) enriched in effector cells. Initial reports demonstrated safety and rapid immune reconstitution using this method, in malignant and non-malignant disorders using several proposed conditioning regimens. No specific

considerations were given to hematologic malignancies. We reviewed a total of twenty seven pediatric patients who underwent haplo-HSCT using  $\alpha\beta$ -T cell depletion between 2013–2016 in a single tertiary referral center. We report the results of 22 procedures performed in eighteen patients transplanted for malignancies. Twenty two haplo-HSCT were performed in eighteen patients. The indication for HSCT was acute leukemia in sixteen (ALL=11, AML=5) and neuroblastoma in two. Median age at HSCT was 5.6 years. Six patients had failed a prior HSCT, and the remainder had no matched donor. The initial reduced-toxicity conditioning regimen consisted of melphalan, fludarabine, thiotepa and ATG, and resulted in a high rate of graft rejections (7 of 9). Thus, a total-body irradiation (TBI)-based regimen was implemented, with prompt engraftment in all the patients. We observed rapid neutrophil and platelet engraftment kinetics (median time to engraft, 10 days and 11.5 days, respectively). Significant treatment related complications were all due to graft failure in patients receiving reduced-toxicity conditioning, with two infection-related mortalities in the presence of prolonged neutropenia. None of the patients developed hepatic sinusoidal-obstruction syndrome, and no grade 3–4 acute graft-versus-host disease (GVHD) or chronic GVHD were observed with either regimen. Importantly, the majority of patients with acute leukemia were free of immunosuppression in the first 100 days post HSCT. The 2-year actuarial event-free and overall survival of the entire cohort were 34% and 55% respectively, with results for TBI-based conditioned patients being 58% and 88%. Overall, we demonstrated that a TBI-based conditioning for haplo-HSCT using  $\alpha\beta$ -T cell depletion for malignant diseases resulted in acceptable outcomes in these high-risk patients without increased toxicity.

**Disclosure of conflict of interest:** None.

#### P421

##### **TECAM (thiotepa, etoposide, cyclophosphamide, Ara-c and melphalan) as conditioning regimen for autologous stem cell transplantation in patients with lymphoma**

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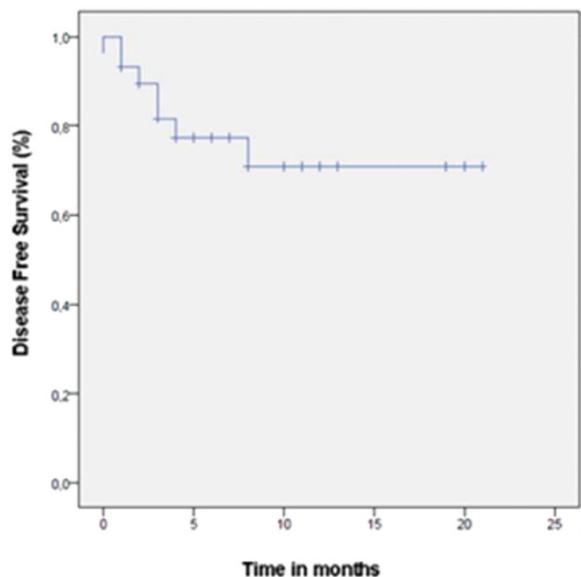
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High-dose chemotherapy conditioning regimens followed by autologous stem cell transplantation (Auto-SCT) generally provide good results in relapsed and refractory lymphomas. We evaluated the efficacy and safety of TECAM regimen as conditioning with autologous stem cell support in patients with relapsed/refractory lymphomas. Thirty-two (16 patients were refractory, 15 patients were relapse and one frontline treated) patients (21 M, 11 F) with lymphoma at various stages (Stage II, 19%; Stage III, 22%; Stage IV, 59%) who underwent ASCT were included in this retrospective study. The median age at transplantation was 52.5 years (range, 28–69 years). The diagnosis were as follows: 9 diffuse large B-cell non-Hodgkin lymphoma (NHL), 9 Hodgkin lymphoma (HL), 5 mantle cell lymphoma, 3 follicular lymphoma, 3 marginal zone lymphoma and 3 T-cell NHL. All patients received TECAM as conditioning regimen that consist of thiotepa (40 mg/m<sup>2</sup> × 4 days), etoposide (200 mg/m<sup>2</sup> × 4 days), cyclophosphamide (60 mg/kg × 1 day), ARA-C (200 mg/m<sup>2</sup> × 4 days) and melphalan (60 mg/m<sup>2</sup> × 2 days). Median CD34(+) cells were 6.7 × 10<sup>6</sup>/kg (range; 1.9–19.3 × 10<sup>6</sup>/kg) which were infused at day 0, followed by recombinant human granulocyte colony-stimulating factor (rHuG-CSF) at a dose of 5  $\mu$ g/kg/day. The median time between mobilization and Auto-SCT was 2 months (range; 1–13 months). The median time to recovery of absolute neutrophil and platelet counts independent of transfusion were 11 (range; 9–19) and 14 (range; 10–41) days, respectively. The median stay in hospital was 28 days (range, 11–108 days). Bacterial, sitomegalovirus and invasive fungal infection were detected in 11 (34%), 4 (13%) and 2 (6%) patients, respectively in first 100 days of Auto-SCT. Three

patients (9.3%) died from transplant-related complications. The overall response rate was 75% (22 CR, 68.8%; 2 PR, 6.2%) after Auto-SCT. Relapse developed in 7 patients during median follow-up period of 6.5 months (range; 1–21 months) after Auto-SCT. The 1-year estimated DFS (Figure 1) and OS were 70% and 45%, respectively. No statistical significance was observed for OS and PFS in terms of gender, patient age (< 60 and ≥ 60 years) and NHL and HL lymphoma group ( $P \geq 0.05$ ). The TECAM regimen for Auto-SCT in lymphoma seems to provide encouraging results in terms of response and its good tolerance with acceptable toxicity.

[P421]

Table 1. Patients characteristics		Table 2. Toxic events	
Number	32	Fever of undetermined origin	20
Sex, M/F	22/11	Staphylococcus infections	8
Age (years), median (range)	52.5 (28-69)	Pseudomonas infections	1
NHL	23	CMV infections	4
HL	9	Invasive fungal infection	2
B symptoms present/absent	9/23	Acute typhlitis	3
Up-front (performed in CR1)	1	Atrial fibrillation	2
Refractory	16	Venous thrombosis	2
Relapse	15	Liver enzyme elevation	2



Disclosure of conflict of interest: None.

#### P422

##### Thiotepa-based conditioning regimens in allogeneic hematopoietic stem cell transplantation for patients with myelofibrosis

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Allogeneic hematopoietic cell transplantation (alloSCT) is the only curative treatment for myelofibrosis. However, its widespread use is limited by early non-relapse mortality (NRM). The optimal modalities of the conditioning regimen are a major

unmet clinical need. In an attempt to reduce early NRM, we used a TBF conditioning regimen (thiotepa, busulfan (BU), fludarabine (Flu) and antithymocyte globulin (ATG)). Our aim was to reduce NRM and improve engraftment by using such TBF conditioning. Thirty consecutive patients with a median age of 56 years (range, 32–69) who underwent alloSCT for primary ( $n=18$ ) or secondary ( $n=12$ ) myelofibrosis were included. According to the refined Dynamic International Prognostic Scoring System (DIPSS-plus), patients were stratified as intermediate-1 ( $n=3$ ), intermediate-2 ( $n=6$ ), and high ( $n=16$ ) risk. Five patients had blast transformation. Ruxolitinib was given to 14 patients (47%) prior to alloSCT. Graft source was PBSCs in 26 patients (87%) and BM in 4 patients (13%). Donors were matched related (MRD,  $n=6$ ), unrelated ( $n=19$ ) and haploidentical ( $n=5$ ). Conditioning regimen was TBF in 18 patients (60%). In our historical cohort 8 patients (27%) received FB (Flu, BU, ATG). In addition, 4 patients received a 'TEC-RIC' sequential conditioning (thiotepa, etoposide, cyclophosphamide, and after 3 days rest, Flu, BU and ATG) for blast transformation ( $n=2$ ) or refractory proliferative myelofibrosis ( $n=2$ ). GVHD prophylaxis consisted of cyclosporine (CsA) and mycophenolate mofetil in 26 patients (87%), CsA and short course methotrexate in 3 patients (10%) with ABO mismatch and CsA alone in 1 patient (3%) with MRD. High dose post-transplant Cy (PT-CY) was added in Haplo cases. No significant difference was observed between TBF, FB and TEC RIC patients in terms of age, gender, Karnofsky score, comorbidity index, number of previous treatment line, history of ruxolitinib administration and source of stem cells. Median follow-up was 20 months (range, 3–75). Two TBF patients died of septic shock before engraftment at day +12 and +19 after alloSCT, respectively. One FB patient died of graft failure at day +108 post alloSCT. Median time to neutrophils and platelets (> 20 G/L) recovery was 15 days (range, 9–28) and 20 days (range, 1–55) with TBF, 17 days (range, 14–53) and 18 days (range, 7–50) with FB, and 19 days (range, 15–24) and 14 days (range, 14–58) with TEC RIC. Grade II-IV acute GVHD occurred in 27.8% of TBF patients, 37.5% of FB patients, and 25% of TEC RIC patients ( $P=0.90$ ). Moderate chronic GVHD developed in 1/13 evaluable TBF, 2/7 FB and 0/4 TEC RIC patients. No severe forms of chronic GVHD were observed. At last follow-up, 1 patient relapsed, 12 died and 18 are still alive. Main causes of death were disease progression ( $n=1$ ), infection ( $n=9$ ) and GVHD ( $n=2$ ). NRM at 2 years was 33.3% in TBF patients, 50% in FB patients, and 25% in TEC RIC patients. The 2-year OS were 66.7% in TBF patients, 37.5% in FB patients, 75% in TEC RIC patients, respectively. CD34+ selected stem cell boost without further conditioning allowed to 4 patients for poor graft function, with significant hematological improvement in 3 patients. TBF conditioning regimen seems to be efficient in alloSCT for patients with myelofibrosis and compares favorably with previously published FB regimens. These preliminary results give a rationale to support a prospective evaluation of this platform.

Disclosure of conflict of interest: None.

#### P423

##### Thymoglobuline versus Campath in patients with myeloid disease receiving a reduced intensity conditioning regimen before allogeneic hematopoietic stem cell transplant (HSCT)

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Main complications after HSCT are relapse and graft-versus-host disease. There are several retrospective and randomized prospective studies reporting that in vivo T-cell depletion (TCD) decreases the risk of acute and chronic GVHD (*Lancet*

*Oncol* 2009; *NEJM* 2016). We proposed here to compare the outcome of patients receiving either Thymoglobuline (ATG), a rabbit anti-human thymocyte immunoglobulin or Campath, a recombinant DNA-derived humanized monoclonal antibody directed against CD52. Campath and ATG are both commonly used as in vivo TCD before HSCT, respectively in United Kingdom and France but very few comparing data are available. All consecutive patients with acute myeloblastic leukemia (AML), myelodysplastic syndrome (MDS) or myeloproliferative neoplasia (MPN) who received a reduced intensity HSCT from an unrelated donor transplanted between 2006 and 2015 were included in this study. A propensity score was used to identify and control potential confounding to relate the treatment group to the outcomes. In the matched sample, Cox regression model was used to describe the association between treatment and outcomes. 322 patients have been included. All patients received Fludarabine and Busulfan with either ATG ( $n=153$ ) or Campath ( $n=169$ ). Patients treated by ATG received cyclosporine plus mycophenolate mofetil or methotrexate and patients treated by Campath received cyclosporine alone as GVHD prophylaxis. Comparing patient and transplant characteristics, ATG patients were older (62 vs 60 years), had less often AML (52 vs 69%), had higher disease risk (adverse DRI: 14 vs 9%; poor cytogenetics: 25 vs 11%; high CIBMTR score: 41 vs 28%), were less often in complete remission at time of transplant (62 vs 76%) and were transplanted less often from a mismatched HLA donor (16 vs 26%). Cumulative incidence of sustained engraftment was in 98% and 99% Campath and ATG patients. Time to neutrophil engraftment was longer in ATG patients (19 vs 13 days). Acute GVHD II to IV rate were higher after ATG (44% vs 19%) as well as chronic extensive GVHD (26% vs 13%). Relapse rate was higher after Campath (44% vs 27%). Disease-free survival (DFS) was higher after ATG (53 vs 37%) and the GVHD-free relapse free survival (GRFS) was similar (35% vs 32%). According to the prognostic factors for outcome, a propensity score was developed selecting 234 patients from the original cohort. The estimation of TCD effect was than studied. Relapse risk was higher in patients treated by Campath while there is a non-significant advantage for ATG in DFS (Table 1).

[P423]

Table 1. Estimation of treatment effects on the matched samples

Endpoints	Model	Hazard ratio	95% Confidence interval	P-Value
Overall survival	original	0.98	0.71-1.36	0.92
	matched	0.95	0.67-1.35	0.78
Relapse	original	0.68	0.46-0.99	0.04
	matched	0.54	0.35-0.84	0.0006
DFS	original	0.8	0.6-1.09	0.1562
	matched	0.74	0.53-1.04	0.081
GRFS	original	1.15	0.87-1.5	0.324
	matched	1.26	0.93-1.7	0.131

TCD with ATG or Campath gives similar OS, DFS and GRFS. Severe acute or chronic GVHD is lowered by Campath but the higher relapse risk counterbalances the potential benefit of Campath finally given similar OS. Nevertheless, lower risk disease patient might benefit from Campath while higher risk patients might benefit from ATG.

**Disclosure of conflict of interest:** None.

#### P424

##### Tolerability and safety using fotemustine or thiotepa-based high-dose chemotherapy regimen in Hodgkin and non-Hodgkin lymphoma: a centre report

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High-dose chemotherapy (HDT) with autologous stem cell transplantation is the standard of care for relapsed/refractory

(RR) or high grade non-Hodgkin-Lymphoma (NHL) and Hodgkin-Lymphoma (HL)2. The standard HDT in autologous stem cell transplantation (ASCT) for Lymphoma is carmustine-based HDT using a combination of carmustine, etoposide, cytarabine and melphalan (BEAM); this standard conditioning programme is used by most groups worldwide1. We have designed novels HDT regimens in which carmustine was substituted by an equal dose of fotemustine (FEAM)3 or thiotepa (TEAM) and we compared these two HDT regimens in terms of engraftment times, toxicity, tolerability and frequency of relapse after ASCT. From February 2011 to September 2016 we consider a total of 67 relapsed/refractory patients affected by HL and NHL respectively 18 HL and 49 NHL with different grade of initial disease (grade I-IV) and different response to prior treatments. The FEAM regimen consisted of Fotemustine 150 mg/m<sup>2</sup> on days -7, -6, etoposide 200 mg/m<sup>2</sup> and aracytin 200 mg/m<sup>2</sup> on days -5,-4, -3, -2 and melphalan 140 mg/m<sup>2</sup> on day -1. The TEAM regimen consisted of THIOTEPA 5 mg/Kg on days -7 and -6, etoposide 200 mg/m<sup>2</sup> and aracytin 200 mg/m<sup>2</sup> on days -5,-4, -3, -2 and melphalan 140 mg/m<sup>2</sup> on day -1. All other drugs were administered according to a standard BEAM regimen1. After a day of rest, autologous peripheral blood progenitor cells were infused on day 0, followed by s.c. G-CSF (5 mg/kg) from day 1 of ASCT until 2 consecutive days when the ANC's were > 500 × 10<sup>9</sup>/l3. The primary objectives of the study were to assess the feasibility and safety of the FEAM and TEAM regimens in terms of acute toxicity, grade of mucositis, hemopoietic engraftment and relapse after ASCT. Acute toxicity include chemotherapy-induced nausea and vomiting, diarrhea, hepatotoxicity, nephrotoxicity and infection complication. In all 67 patients CD34+ cells were collected from peripheral blood and the median number of infused cells per patient was 5.79 × 10E6/Kg. The median time of engraftment was 9 days for neutrophil recovery ( $N > 500 \times 10^9/L$ ) and 11 days for Plt recovery ( $> 20\,000 \times 10^9/L$ ). Acute toxicity occurred in 14 total patients (20.8%), mucositis grade 3-4 occurred in 34 patients (50% of cases). Frequency of relapse in all 67 cases was 43.2%. FEAM conditioning regimen was used in 41 cases showing a median time of neutrophil recovery of 10 days and a median time of plt recovery of 11 days. Acute toxicity occurred in 4 of these cases (9.7%), mucositis grade 3-4 occurred in 18 patients (43.9% of cases). Frequency of relapse in FEAM group of patients was 41.4%. TEAM conditioning regimen was used in 26 cases showing a median time of neutrophil recovery of 9 days and a median time of plt recovery of 11 days. Acute toxicity occurred in 10 of these cases (38.4%), mucositis grade 3-4 occurred in 16 patients (61.5% of cases). Frequency of relapse in TEAM group of patients was 50%. Relapse/progression of lymphoma and conditioning regimen toxicities remain limitations to treatment success. The two novels HDT regimens FEAM and TEAM are safe and feasible and show similar engraftment times, tolerability and frequency of relapse. Maybe the TEAM regimen shows toxicity slightly higher than FEAM regimen but longer follow-up is needed to evaluate fully its efficacy and long-term safety.

**Disclosure of conflict of interest:** None.

#### P425

##### Toxicological side effects of fludarabine and treosulfan conditioning before allogeneic stem-cell transplantation

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Treosulfan is a prodrug of a bifunctional alkylating cytotoxic agent. There are few reports regarding toxicological side effects of treosulfan-based conditioning prior to HSCT. Here we report on incidence of early potential treosulfan-related toxicity in 118

patients treated with treosulfan-based conditioning before HSCT. Treosulfan was given at a dose of 14 g/m<sup>2</sup>/d for 3 days in combination with fludarabine 30 mg/m<sup>2</sup>/d for 5 days prior to HSCT. Most patients (n=93) had a haematological malignancy, while 25 patients had a non-malignant disorder as HSCT indication. An HLA-A, -B and -DR matched unrelated donor (MUD) was used in 80 cases, 33 patients had a HLA-identical sibling donor and 5 received an HLA-A, -B or -DR allele mismatched unrelated donor. As graft versus host-disease (GVHD) prophylaxis, most patients (n=93) received cyclosporine and methotrexate. Patients medical records were scrutinized retrospectively to collect laboratory tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine) before HSCT and then weekly until 5 weeks after HSCT. Levels of AST and ALT were significantly increased 1 week after HSCT compared to before HSCT. However, only a few patients had transaminase levels over 2 or 3 times the upper normal level (UNL) levels decreased sharply after the first week. Most of the cases with high levels of AST/ALT at one week had normal or close to normal levels before HSCT. Creatinine levels increased after week 2 but no patient had levels  $\geq 2 \times$  UNL. Clinical features of all oral mucositis (OM) were recorded using the World Health Organization (WHO) scoring system. Most patients (68%) had no or very limited (grade I) OM, 22% had grade II and 10% had grade III or IV of OM. According to our toxicological results this is low-toxic protocol. However, all patients became neutropenic, 61% already at the time of graft infusion, indicating that the protocol has a myelo-toxic effect comparable to conventional MAC protocols. All patients engrafted, except three patients who died very early. Median time to neutrophil and platelet engraftment was 18 (range 10–31) and 15 days (9–55), retrospectively, which is significantly later when compared to engraftment data for other RIC protocols used at our centre (data not shown). Median duration of neutropenia ( $< 0.5 \times 10^9/L$ ) was 17 days (5–31), comparable to what is expected after conventional MAC conditioning. Secondary graft failure (GF) occurred in 8 (6.8%) patients, all having a non-malignant disorder and 6/8 having a URD. Non-relapse mortality (NRM) was 7.5% (95% CI 3.7–13.3%) at 100 days and 11.9% (6.8–18.5%) at one year after HSCT. Causes of death within one year after HSCT were: relapse 7, Epstein-Barr virus associated post-transplant lymphoproliferative disease (PTLD) 4, other infections 4, organ failure 2, GVHD 2, hemophagocytic lymphohistiocytosis (HLH) 1. Other infections occurring within 100 days after HSCT were cytomegalovirus (CMV) reactivation 46 (39%), invasive fungal infection 6 (5.1%) and blood stream infection 47 (40%). Veno-occlusive disease of the liver or sinusoidal obstruction syndrome (VOD/SOS) occurred in one patient and haemorrhagic cystitis in two patients. This study shows that early regimen-related toxicity after HSCT was low despite similar marrow toxicities compared to MAC regimens.

**Disclosure of conflict of interest:** None.

#### P426

### Treosulfan, fludarabine and 4Gy TBI conditioning with ATG and sirolimus-based GvHD prophylaxis (TrRaMM4Gy) for haploidentical stem cell transplantation in patients with hematological malignancies

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Allogeneic stem cell transplantation from haploidentical donors (haploSCT) is an increasingly adopted option for patients (pts) with high-risk hematological malignancies. In our Institution, we previously described a platform for unmanipulated peripheral

blood stem cell (PBSC) haploSCT using a calcineurin-free GvHD prophylaxis with sirolimus, micophenolate and anti-human T-lymphocyte immunoglobulin (ATG) after conditioning with Treosulfan and Fludarabine (TrRaMM; Peccatori *et al.*, *Leukemia* 2015). As an attempt to decrease relapse rate, especially in advanced-phase diseases, we designed a new phase II prospective clinical trial intensifying conditioning regimen with the addition of 4Gy total-body irradiation (TBI) (TrRaMM4Gy; EudraCT#2011-001534-42). We report results on 75 pts. 75 pts affected by AML (n=49), other myeloid (n=8) and lymphoid (n=18) malignancies were prospectively enrolled from May 2010 to June 2015. Median pts age was 45 y (range 17–67). Revised disease risk index (R-DRI) was low or intermediate in 31 pts, high in 34 pts and very-high in 10 pts. Twenty-five pts had previously received an allogeneic stem-cell transplantation with a median time from 1st to 2nd SCT of 17 months (3–81). Median HCT-comorbidity index by Sorror *et al.* was 1 (0–5). Pts received a myeloablative conditioning regimen consisting of Treosulfan (14 g/m<sup>2</sup>/d from -6 to -4), Fludarabine (30 mg/m<sup>2</sup>/d from -6 to -2) and TBI 4Gy (fractionated in 2 doses, from -1 to 0). Source of stem cells were unmanipulated G-CSF-mobilized PBSC from haploidentical donors. GvHD prophylaxis consisted of ATG-Fresenius (Grafalon, Neovii) 10 mg/Kg/d from -4 to -2, Rituximab 200 mg/m<sup>2</sup> on -1, mycophenolate mofetil 10 mg/kg from -1 to +30 and sirolimus (target concentration 8–15 ng/mL) from -7. Median infused CD34+ and CD3+ cell doses were  $6.9 \times 10^6/Kg$  and  $2.15 \times 10^8/Kg$ , respectively. Median follow-up for survivors was 48 months (5–74). Neutrophil engraftment occurred in 95% of pts with a median of 17 d (9–47), platelet engraftment was reached in 76% of pts with a median of 17 d (4–22). The 100-d cumulative incidence (CI) of grade  $\geq 2$  acute GvHD (aGvHD) was  $31 \pm 10\%$  and of grade  $\geq 3$  aGvHD  $27 \pm 10\%$ ; the 2 years CI of chronic GvHD was  $34 \pm 11\%$ . The CI of transplant-related mortality (TRM) at 1 y and 4 y were  $31 \pm 10\%$  and  $34 \pm 11\%$ , respectively. The CI of relapse at 1 y and 4 y were  $27 \pm 10\%$  and  $34 \pm 11\%$ , respectively, with a median time to relapse of 90 d (5–1426). Interestingly, we did not observe any extramedullary relapse; loss of mismatched HLA-haplotype occurred in 33% of relapses. Among 36 pts who were in active disease at time of haploSCT and who were evaluable, 94% achieved complete remission (CR) and full donor chimerism at day+30. The 1y and 4y probabilities of disease-free survival (DFS) were  $42 \pm 11\%$  and  $33 \pm 11\%$ , respectively. At 4 y, 27% of pts are alive, disease-free and immunosuppression-free. Median time to sirolimus withdrawal was 4.4 months (0.7–36). R-DRI was significantly associated with DFS (at 4 y, 44% if low-intermediate, 34% if high, 10% if very high,  $P=0.01$ ). The addition of 4 Gy TBI to Treo-Flu induced a CR and full engraftment in advanced-disease and abolished extramedullary post transplant relapse of high risk leukemias. Although DFS is satisfactory in such high risk population, high rates of severe aGvHD and cGvHD call for further improvements in GvHD prophylaxis.

**Disclosure of conflict of interest:** None.

## Minimal residual disease, tolerance, chimerism and immune reconstitution

#### P427

### 6-color-based HLA-Flow method for the monitoring of minimal residual disease and chimerism after HLA-mismatched allogeneic hematopoietic cell transplantation in children

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The number of human leukocyte antigen (HLA)-mismatched hematopoietic cell transplantation (HCT), including cord blood transplantation, has been increasing. HLA-Flow method can discriminate mismatched HLA antigens between donor and recipient by using flow cytometry, and can evaluate minimal residual disease (MRD) or chimerism after HLA-mismatched HCT. By developing more simple methodology, HLA-Flow might be more widely applicable. We have developed modified 6-color-based HLA-flow method. The aim of this study is to evaluate the utility of the 6-color-based HLA-flow for monitoring of MRD and chimerism after HLA-mismatched HCT in children. From June 2013 to November 2016, serial monitoring of MRD or chimerism by the 6-color-based HLA-Flow was performed in twelve patients undergoing HLA-mismatched HCT (46 tests). Nucleated cells obtained from bone marrow were stained by immunofluorescent antibodies against HLA antigens mismatched between donors and recipients. These cells were also stained by immunofluorescent antibodies against surface antigens such as CD3, CD19, CD33, CD34 and CD16/CD56 for determining lineage of the cells. These surface antigens were also used as a marker of leukemic blasts in the MRD study. We used 6-color-based flow cytometry (FACS-Navios) and the data were analyzed with Flow Jo. Erythroblasts and dead cells were excluded from the analysis. In each study, at least 105 cells were analyzed. For MRD analysis, we concurrently tested real-time quantitative polymerase chain reaction (PCR) of peripheral WT1 mRNA or leukemia-specific fusion genes. PCR of polymorphic short tandem repeats or fluorescent *in situ* hybridization of X/Y chromosomes was concurrently tested for chimerism study. Age of patients ranged from 0 to 16 years. Donor sources included bone marrow ( $n=9$ ) and cord blood ( $n=3$ ). For MRD monitoring of acute leukemia ( $n=9$ ), the 6-color-based HLA-Flow could detect MRD in three patients. Five patients have not experienced relapse. No discordance with other MRD markers was observed in these patients. HLA-Flow could not separate donor-derived cells from recipient-derived ones in one patient receiving bone marrow transplantation. As for chimerism testing ( $n=3$ ), the 6-color-based HLA-Flow could successfully evaluate quantitative lineage-specific chimerism in all patients. There is no discrepancy between HLA-Flow and other methods. We could complete evaluation of the 6-color-based HLA-Flow within two days in all tests. The 6-color-based HLA-Flow is a simple, quick and useful method for the quantitative evaluation of MRD and lineage-specific chimerism after HLA-mismatched HCT in children, irrespective of donor sources. It is thought that our method is applicable in all institutions owing 6-color-based flow cytometry.

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**Disclosure of conflict of interest:** None.

#### P428

### Cytomegalovirus infection/reactivation promotes faster and stable CD8+ cells immune reconstitution and improves survival in acute myeloid leukemia: a single center experience on 122 patients

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Allogeneic Haematopoietic Stem Cell Transplantation (allo-HSCT) is still the only immunotherapeutic procedure able to cure high risk Acute Myeloid Leukemia (AML). Post-transplant immune reconstitution (IR) and CMV infection/reactivation (CMVi/r) remain the two most important factors affecting the success of the procedure. Thus, the present study was aimed at evaluating the impact of IR and CMVi/r on Overall Survival (OS), Progression Free

Survival (PFS), Cumulative Incidence of Relapse (CIR) and acute/chronic GVHD incidence in AML patients (pts) submitted to allo-HSCT at our Institution between January 2003 and December 2014. This retrospective study evaluated 122 AML pts submitted to allo-HSCT from 56 matched sibling donors (MSD) and 66 matched unrelated donors (MUD) who provided bone marrow (BM) or peripheral blood as stem cell grafts. IR was evaluated at 100, 180 and 365 days post-transplant in all 122 pts. CMV-DNA copies were determined in peripheral blood by quantitative PCR twice weekly in the first 100 days post-transplant and subsequently once weekly. CMVi/r was analyzed as a time-dependent covariate. Effect of CMVi/r on OS and PFS was estimated by Cox proportional hazard model. CIR and GVHD incidence were analyzed with a competing risk approach, considering death from any cause as a competing event. Effect of CMVi/r on CIR and GVHD were evaluated by Fine & Gray model. Median age at allo-HSCT was 50.7 years (19.1–68.9). In our population 68% of donors were seropositive for a previous CMV infection and pts transplanted from these donors showed a significantly lower cumulative incidence of CMVi/r than pts transplanted with seronegative ones (sHR=0.56, 95%CI:0.33–0.94,  $P=0.029$ ). CMVi/r had a strong effect on IR: CD8+ cell numbers at 180 ( $P<0.001$ ) and 360 days ( $P<0.001$ ) post-HSCT were significantly higher in pts with a CMVi/r than in those without such an event. As transplantation outcomes are concerned, pts who developed a CMVi/r during the post-transplant period had a significant better OS [HR=0.45, (0.26–0.77),  $P=0.004$ ] (Fig.1) and PFS [HR=0.42, (0.25–0.70),  $P=0.001$ ] and a significantly lower CIR [sHR=0.32, (0.15–0.65),  $P=0.002$ ] than those without it. Worth of noting is the fact that the progressive increase in CD8+ cell numbers during the post-transplant period had no effect on acute and chronic GVHD incidence: pts who developed and those who did not developed a CMVi/r presented a similar frequency of aGVHD and cGVHD [sHR=0.74 (0.32–1.73)  $P=0.49$ ] and [sHR=1.18, (0.73–1.91),  $P=0.489$ ]. Furthermore and of more relevance was the fact that at 6 months post-HSCT in pts with a CMVi/r a higher CD8+ cell number correlated with a significantly reduced risk of relapse [HR:0.98, (0.97–0.99),  $P=0.002$ ] and the significantly higher NK cell number seems to be protective against cGVHD [HR:0.99, (0.98–0.99),  $P=0.045$ ]. No deaths for CMV disease were registered. Our study demonstrates that CMVi/r influences the success of allo-HSCT by determining a better IR characterized by a higher CD8+ cell number that might exert an immune protective control on disease outcome by improving OS, PFS and CIR with no effect on GVHD. Another factor of utmost importance to achieve this same goal might be constituted by the significantly increased NK cell number six months after allo-HSCT.

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[P428]

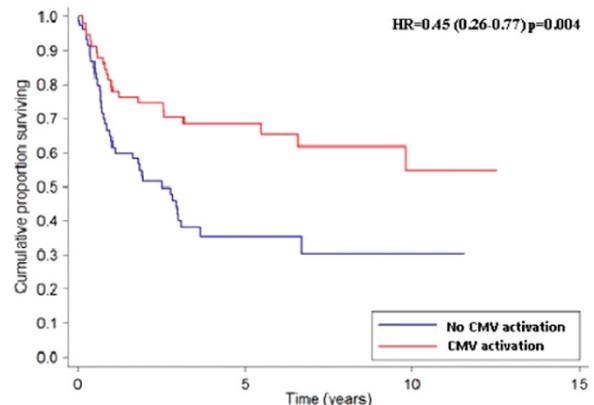


Fig. 1. OS in relation to the occurrence of a post-transplant CMV infection/reactivation

**Disclosure of conflict of interest:** None.

**P429**  
**Dynamics of molecular response in AML patients with NPM1 and FLT3 mutations undergoing allogeneic stem cell transplant**

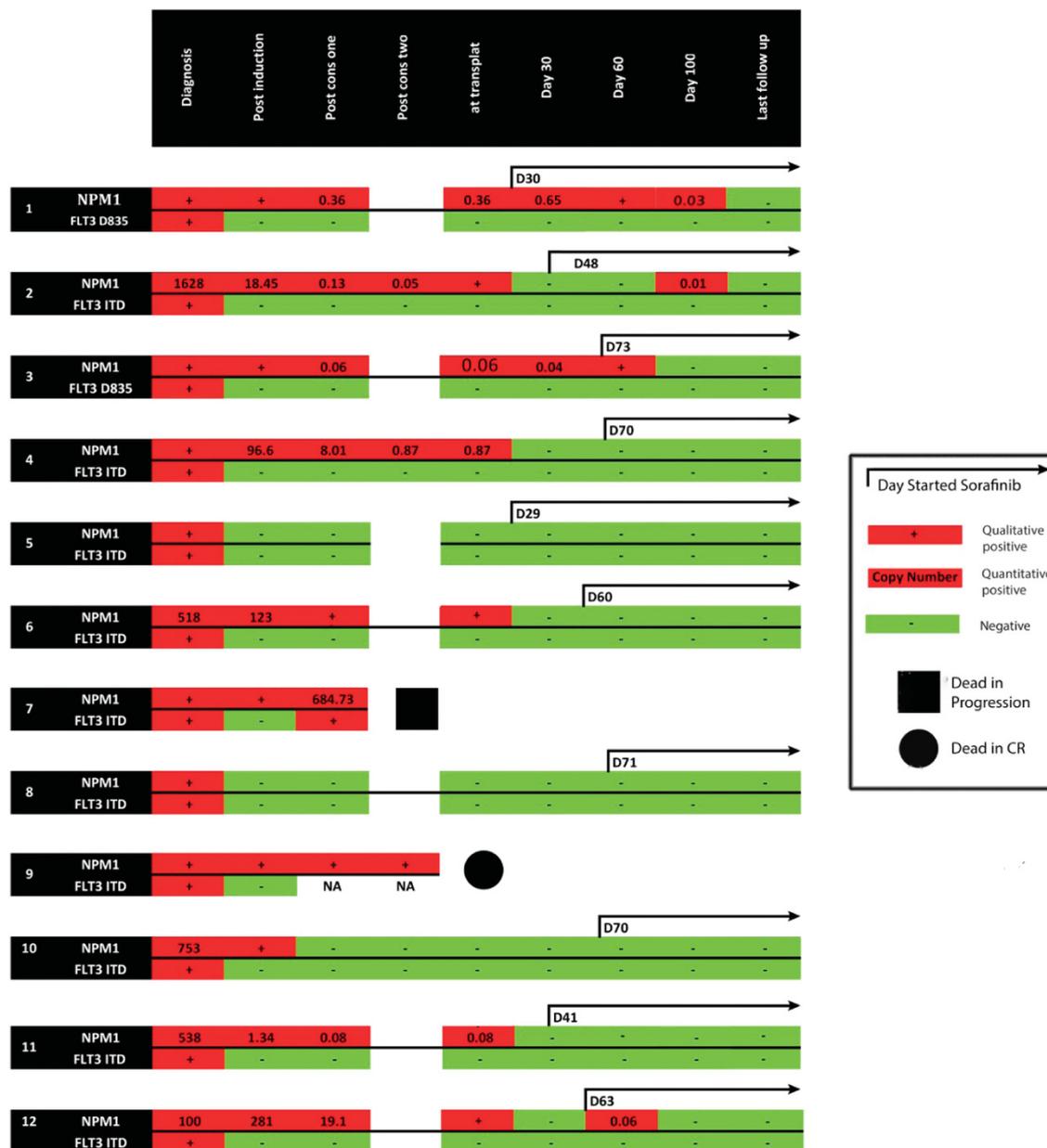
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Concomitant NPM1 and FLT3 mutation occurs in 20% of AML patients. Molecular response and achievement of negative minimal residual disease (MRD) are strong predictors of long-term outcome. However, little is known about the dynamics of molecular response in NPM-1 and FLT-3 double positive

mutations. To assess the dynamics of molecular response to treatment in AML adult patients with concomitant FLT3 and NPM1 mutations. This retrospective single center study was approved by the institutional review board of American University of Beirut Medical Center. Twelve consecutive newly diagnosed ( $n = 11$ ) or relapsed ( $n = 1$ ) AML patients received Idarubicin/cytarabine induction and one or two consolidation (s) (Table 1). Seven patients received allogeneic stem cell transplant (allo-SCT) and 3 had haploidentical-SCT (haplo\_SCT); all followed by post-transplant sorafenib maintenance. Median follow-up was 11.5 (6–27) months. All transplanted patients remain alive and disease free. FLT3 mutation was tested on DNA using a qualitative method with a sensitivity of 0.01%. NPM-1 mutation was tested on cDNA using a qualitative or a quantitative RT-PCR with a sensitivity of 0.01% and 0.008 NCN respectively. Patients were tested at diagnosis, after induction, after each consolidation, before and

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at days 30, 60 and 100 after allo-SCT for Kinetics of NPM1 and FLT3 molecular response. After induction, FLT3 became negative in all tested patients ( $n=10$ ). After first consolidation, FLT-3 was not tested in 3 patients who had a negative result after induction, was negative in 8 patients including the 2 patients who were not tested after induction, whereas a molecular relapse was noted in one patient who developed a hematological relapse and rapidly died. Another patient died after the third consolidation, in complete remission, due to septic shock. No molecular positivity for FLT-3 was noted later on, whether after second consolidation or post-transplant. Conversely, NPM-1 mutation became negative in 2 out of 12 tested patients after induction, in 1 additional patient after first consolidation and in 7 additional patients after SCT, mostly after starting sorafenib. NPM-1 MRD value remained elevated in 3 out of 4 patients with quantitative assessment at diagnosis and post induction (Figure1). FLT3 become negative early after induction while NPM1 negativity lags behind. Persistent NPM-1 MRD does not seem to predict post-transplant outcome and may indeed become negative after sorafenib. These results need confirmation in larger studies.

**Disclosure of conflict of interest:** None.

#### P430

##### **Early immune cells subset associated with outcomes after allogeneic stem cell transplantation in childhood patients suffering acute leukemia and myelodysplastic syndrome**

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In allogeneic stem cell transplantation (allo-SCT), an early detection of the transplant outcomes such as overall survival (OS), event-free survival (EFS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) is fundamental regarding the use in time of additional therapy after SCT. Therefore, we investigated the association between early immune reconstitution (IR) on day +30 after allo-SCT and outcomes in children suffering from acute leukemia or myelodysplastic syndrome (MDS). This study collected data from 188 allo-SCT from January 2005 until December 2014 in our institution. The median survival follow-up was 38 months. Indications of allo-SCT were ALL ( $n=113$ , 60%), AML ( $n=44$ , 23%) and MDS ( $n=31$ , 17%). The median age was 10 years (range, 0.6 - 18). Patients were transplanted in CR ( $n=131$ , 70%) and PR/NR ( $n=57$ , 30%). Patients included in the study received 1st SCT ( $n=170$ , 90%), 2nd SCT ( $n=15$ , 8%) or > 2 SCT ( $n=3$ , 2%). Grafts were from sibling (MSD;  $n=42$ , 22%), matched unrelated (MUD;  $n=95$ , 51%), haploidentical ( $n=45$ , 24%) or mismatched unrelated (MMUD;  $n=6$ , 3%). Conditioning regimens were TBI-based ( $n=87$ , 46%) or chemo-based ( $n=101$ , 54%). Stem cells were from bone marrow ( $n=118$ , 63%) or peripheral blood ( $n=70$ , 37%). We analyzed the absolute count of lymphocytes (ALC), monocytes, CD3+ T cells, CD3+CD4+ T-helper cells, CD3+CD8+ cytotoxic T-cells, CD3-CD56+ natural killer (NK) cells and CD19+ B cells assessed on day 30 ± 5 after SCT. We used the percentiles of the lymphocyte subsets of the same cohort to categorize the samples throughout the study. Patients with ALC over the 50th percentile of ALC (ALC < 327 cells/ $\mu$ l) had a 1.97-fold increased hazard ratio (HR) to develop relapse ( $P=0.017$ ). NK cell counts on day 30 after SCT were strong associated with OS, EFS, CIR and NRM. Patients with NK cell count over the 75th percentile (NK > 268.8 cells/ $\mu$ l) had increased HR for OS (HR=1.8,  $P=0.01$ ) and EFS (HR=2.01,  $P=0.017$ ) compared to patients with NK count under the 75th percentile. Patients with NK cells over the 25th percentile (NK < 52.5 cells/ $\mu$ l) had a HR=3.3 ( $P=0.009$ ) for relapse and HR=0.364 ( $P=0.016$ ) for

NRM compared to patients with NK cell count under the 25th percentile. Monocyte cell count on day 30 correlated with OS, EFS and CIR. Patients with CD14+ cells count under the 15th percentile of CD14+ (CD14+ < 242 cells/ $\mu$ l) has an increased hazard ratio for OS, EFS and relapse compared to patients with CD14+ cell counts over the 15th percentile. No association between absolute cell count of CD3+, CD4+, CD8+ and CD19+ on day +30 after allo-SCT and any outcomes either OS, EFS, CIR or NRM was found. The study confirms the strong association between early IR and outcomes after allo-SCT in children. Our study suggests that especially NK cell and monocyte cell count on day +30 may have significant prognostic implications. Our findings suggest that the cells count of ALC, NK cells and monocytes on day +30 after allo-SCT could be useful to predict outcomes after allo-SCT and should be taken into account in considering alternative treatment.

**Disclosure of conflict of interest:** None.

#### P431

##### **Early immune reconstitution impact on overall survival after haploidentical hematopoietic stem cell transplantation**

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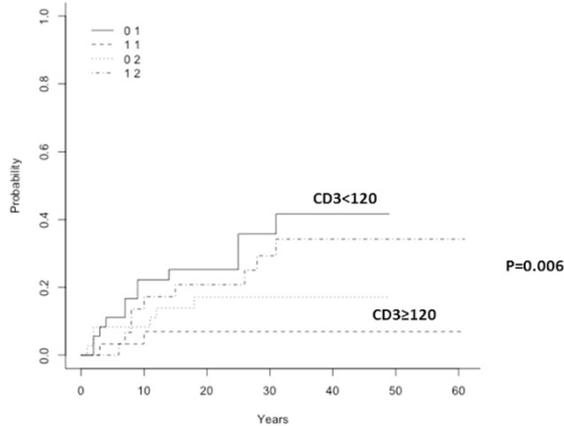
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Early immune reconstitution (EIR) has proven to be a significant determinant for the outcome of allogeneic hematopoietic stem cell transplantation. In the setting of unmanipulated haploidentical transplantation (Haplo-HSCT), some groups have identified the absolute leukocyte count on day +30 (ALC30) as an independent prognostic factor in terms of overall survival (OS), disease free survival (DFS) and infectious mortality (IM). The aim of this study was to evaluate the impact of EIR on OS, DFS and IM among patients who underwent Haplo-HSCT with posttransplant cyclophosphamide (PTCy) at our institution. From July 2011 to April 2016, 83 Haplo-SCT were performed at our institution. Threedied before day 30 after Haplo-SCT, and 13 patients had missing data. Conditioning regimen consisted of fludarabine, cyclophosphamide and busulfan. Twenty-nine patients received a reduced intensity conditioning regimen (1–2 days of busulfan) while 37 a myeloablative regimen (3–4 days of busulfan). GVHD prophylaxis comprised PTCy, cyclosporine and mycophenolate mofetil. Patients were assessed for EIR by means of ALC30, CD3+ T lymphocyte count on +30 (CD3), NK lymphocyte count on +30 (NK) and NK CD56<sup>bright</sup> percentage on +30(CD56<sup>br</sup>). We analyzed 66 pts, with a median follow-up of 21 months (9–36). The median age of the pts was 43 (range 30–57), 76%men. Diagnosis were: AML(32%), HL (23%)non-HL (17%), ALL (8%),MDS(8%), CML(6%), others(6%). 55% were in complete remission at the time of transplant, 21% in partial remission and 24% had overt disease. In terms of infectious complications, CMV reactivation was documented in 76% of the pts, 1% developed a proven invasive fungal infection and 26% suffered from BK+hemorrhagic cystitis. Median OS and DFS were 21 (9–36) and 17 months (7–31), respectively. IM rate was 21% at the end of follow up. Median follow-up was 21 months (9–36). ROC curves were used to determine the optimal cut-off values for each of the studied parameters: 300 cells/ $\mu$ l for ALC30, 120 cells/ $\mu$ l for CD3, 41 cells/ $\mu$ l for NK and 83% for CD56<sup>br</sup> were chosen. Pts with ALC30  $\geq$  300/ $\mu$ l had better OS ( $P=0.005$ ) and DFS ( $P=0.05$ ), than those with ALC30 < 300/ $\mu$ l. Median OS and DFS were 25 months vs not reached (NR) and 22 months vs NR, respectively. Pts with CD56<sup>br</sup>  $\geq$  83% had better OS ( $P=0.04$ ) than those with lower values. Median OS was 25 months vs NR; however no difference was seen in terms of DFS. We didn't observe statistically significant differences in OS or DFS, among pts with different levels of CD3 and NK on +30. Cumulative

incidence of IM was significantly lower in pts with an ALC30  $\geq$  300 ( $P=0.04$ ), pts with CD3  $\geq$  120/ $\mu$ L ( $P=0.006$ ) and pts with NK  $\geq$  41 ( $P=0.04$ ); patients with CD56<sup>br</sup>  $\geq$  83% showed tendency to have lower cumulative incidence of infectious mortality ( $P=0.24$ , non-significant). Cumulative incidence of relapse was not affected by ALC30, CD3, NK or CD56<sup>br</sup>. Our study supports the independent prognostic value of early immune reconstitution after unmanipulated haplo-identical transplantation with PTCy, especially in terms of lower infectious mortality. OS and DFS were better among patients with ALC30  $\geq$  300 cells/ $\mu$ L. Pts with CD56<sup>br</sup>  $\geq$  83% also showed better OS. No correlation was found between CD3 or NK on +30 with OS or DFS. Cumulative incidence of infectious mortality was affected by ALC30, CD3 and NK on +30; while CD56<sup>br</sup> seems to have less impact.

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Cumulative incidence of infectious mortality based on CD3 at day 30



Disclosure of conflict of interest: None.

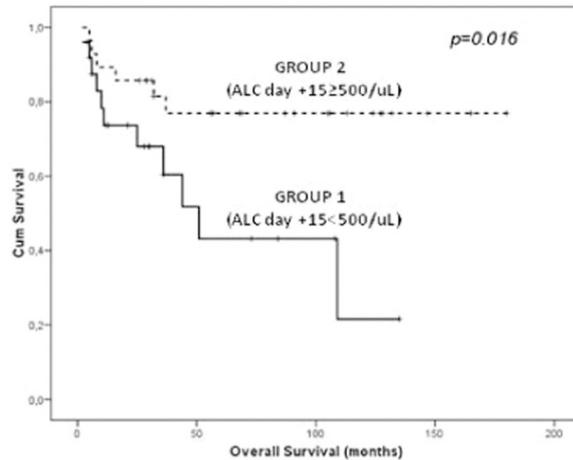
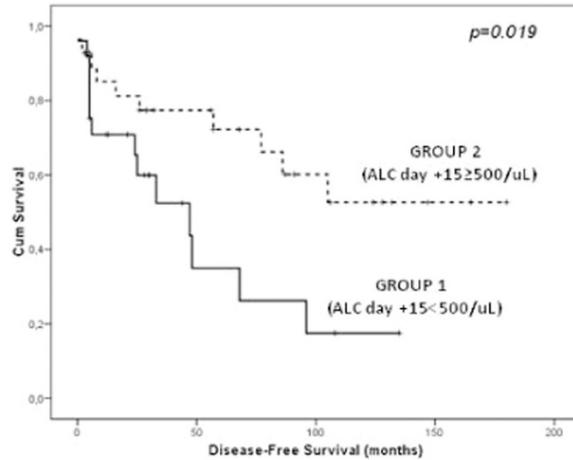
**P432**  
**Early lymphocyte recovery predicts prolonged survival after autologous stem cell transplantation for non-Hodgkin lymphoma**

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An early absolute lymphocyte count (ALC) recovery after autologous stem cell transplantation (ASCT) for hematologic malignancies has been related with an improved transplant outcome due to a faster autologous immune restoration. In this retrospective study we analyze post-transplant survival of Non Hodgkin lymphoma (NHL) patients and its relation with ALC at day +15 post-ASCT. We analyzed 53 consecutive adult NHL patients who underwent ASCT at the Hematology and SCT Department of Hospital Maciel (Montevideo, Uruguay). Only individuals with at least 6 months post-transplant follow up were included. All patients received BEAM (BCNU, Etoposide, Cytarabine and Melphalan) conditioning regimen followed by peripheral blood stem cells previously collected by apheresis. Median CD34+ cell dose was  $4.13 \times 10E6/kg$  (1.62–12.58). Median ALC at day +15 was 500/ $\mu$ L. Patients were divided into two groups: ALC at day +15 inferior than 500/ $\mu$ L (group 1) and ALC at day +15 superior or equal than 500/ $\mu$ L (group 2). Differences between groups were analyzed using t-Student and Chi-square tests, with statistical significance determined at  $P < 0.05$ . Disease free survival (DFS) and overall survival (OS) were analyzed by Kaplan Meier method. Differences in survival between groups were determined by log-rank test. No differences were observed between groups regarding gender, histology, disease status at transplant and

cell dose. Patients in group 1 were older and more heavily pre-treated. Neutrophils and platelets engraftment were significantly faster in group 2 (Table 1). After a median follow up of 36 months, disease-free survival (DFS) and overall survival (OS) were superior in group 2. Median DFS was 47 months and not reached ( $P=0.019$ ) and OS was 51 months and not reached ( $P=0.016$ ) in groups 1 and 2 respectively (Figure 1). An early ALC recovery after ASCT was associated with a superior DFS and OS in NHL patients. Individuals with ALC major or equal than 500/ $\mu$ L had also a shorter time to neutrophils and platelets recovery and a shorter hospital stay. In this study, CD34+ cell dose does not seem to be a determinant factor for lymphocyte recovery. The load of previous treatment may influence lymphocyte recovery after ASCT. These results support the association between early post-ASCT lymphocyte recovery and improved survival in NHL patients.

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**P433**  
**Impact of day 100 bone marrow chimerism and subsequent donor lymphocyte infusion on survival in patients with AML/MDS**

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T cell depletion (TCD) reduces the risk of graft versus host disease (GVHD) but also the graft versus leukaemia (GVL) effect, thus increasing the risk of relapse. Donor lymphocyte infusions (DLI) can be given to boost donor chimerism, with the intention of enhancing the GVL effect.<sup>1</sup> It is not currently known whether giving DLI based on bone marrow chimerism (BMC) influences survival, or whether certain groups of patients benefit more from DLI than other groups. In addition, it is not known whether the overall aim of achieving 100% BMC associates with improved survival. We investigated whether Day 100 (D100) BMC was predictive of survival, and whether giving DLI based on this result was associated with improved overall survival. Data were retrospectively collected from case notes and laboratory reports for patients who underwent allogeneic stem cell transplant (alloSCT) for AML or MDS at the Northern Centre for Bone Marrow Transplantation between 2010 and 2015. Patients who died prior to D100 were excluded from the analysis. Of the 147 patients analysed (AML 117, MDS 30), 68% were male and 38% female. The median age was 59 years (range 22–74). Conditioning was with Flu/Bu/Alemtuzumab (63), Flu/Mel/Alemtuzumab (45), Cy/TBI Alemtuzumab (7), FLAMSA TBI/Bu ATG/Alemtuzumab (27), other (5). 103 (70%) received a graft from an unrelated donor, 42 (29%) a matched sibling donor and 2 (1%) another source. 143 (97%) received mobilised PBSCs, 3 (2%) bone marrow and 1 (1%) cord blood. Statistics were performed using GraphPad Prism. *P* values were calculated using the Chi square test and taking *P* < 0.05 to determine significance. BMC was divided into 3 groups 100%, 90–99% and <90%. 100% BMC at D100 was associated with a significant increase in 2 year overall survival (OS) (74.6% vs 57.3% and 33.4% for 90–99% and <90%, respectively, *P* < 0.0012). Patients with a D100 BMC < 80% had a 2 year OS of < 10% (with relapse the cause of death in 90%). In patients whose D100 BMC was < 90%, there was a significant improvement in 2 year OS seen in those who received DLI (61.7% survival at 2 years vs 0% with no DLI, *P* < 0.0026) (Figure 1: OS by D100 BM chimerism (with and without DLI). Attainment of 100% BMC at a subsequent time point also significantly improved survival in those with a D100 BMC of 90–99% (79.4% 2 year survival vs 33.6% who never attained 100%, *P* < 0.001) and < 90% (100% 2 year survival vs 14.7%, *P* < 0.006). We found D100 BMC to be predictive of OS in this population. In addition, DLI was associated with an improvement in OS, especially in patients whose BMC at D100 was < 90%. There was also a statistically significant improvement in OS seen in patients who subsequently attained a 100% BMC, where it was < 100% at D100.

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**Disclosure of conflict of interest:** None.

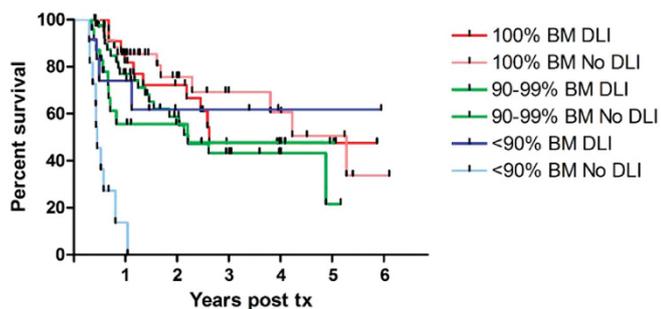
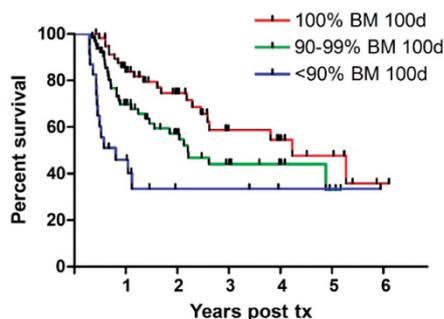
#### P434

### Impact of early lymphocyte recovery and infused allograft characteristics on relapse in allogeneic transplant recipients for acute leukemia

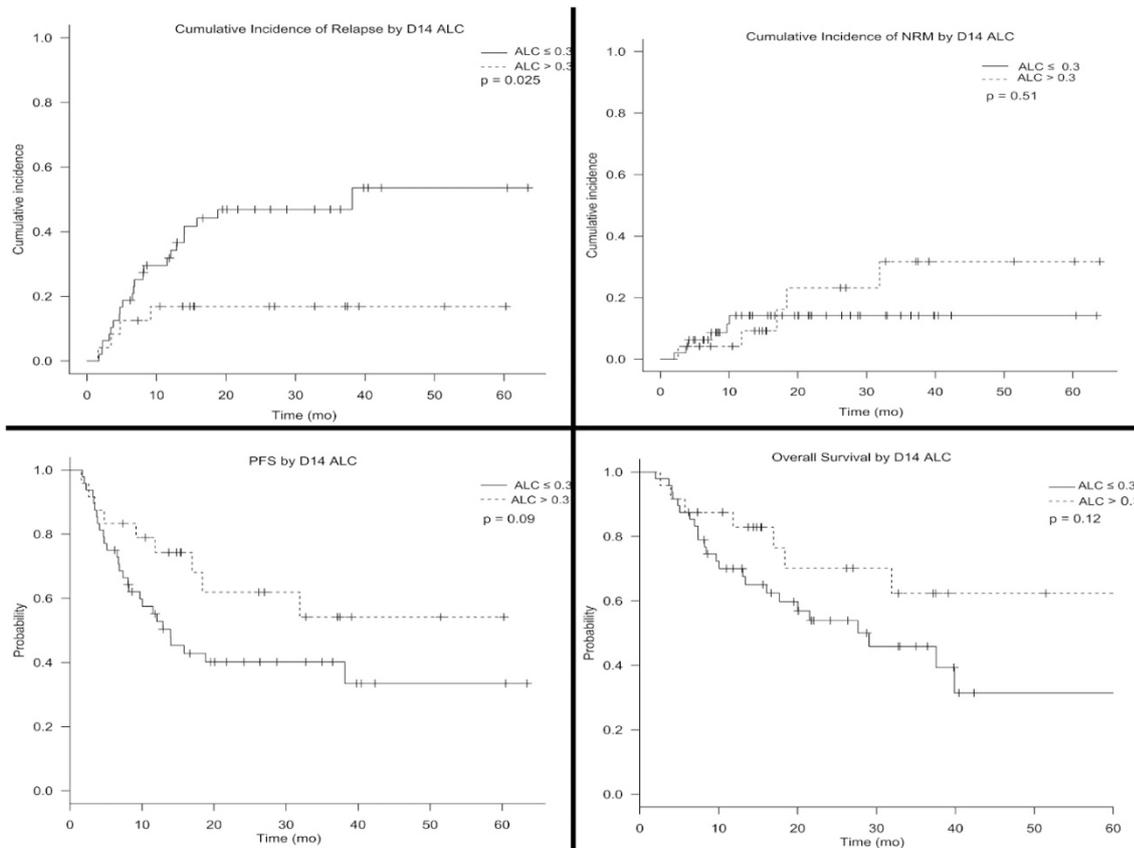
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Early absolute lymphocyte count (ALC) recovery has been reported to improve relapse rate in allogeneic transplant (HCT) recipients for acute leukemia (Kumar *et al.* 2001, 2003, Le Blanc *et al.* 2009). However, there is heterogeneity in published literature regarding timing and threshold of ALC. The objectives of this analysis were to examine the optimal ALC recovery cutoff utilizing receiver operator characteristics (ROC) analysis and to examine infused allograft characteristics associated with early ALC recovery. After due IRB approval, patients (pts) with AML and ALL who underwent HCT at our institution between 2010–2015 were identified. Pts with T-cell depletion or maintenance post HCT were excluded. Data were collected retrospectively from the patient's records. Cellular contents of infused products (CD34, CD3, TNC, MNC, ALC and AMC) in addition to ALC post HCT were analyzed and optimal cutoff, if present, was established using ROC analysis for the end point of relapse. Time to end point analysis was computed using the Kaplan–Meier with log ranks. For competing events, cumulative incidence was computed using Grey's model. Univariable and multivariable analyses were performed using Cox proportional hazard regression. A total of 72 pts met the inclusion criteria and were analyzed. Optimal ALC cutoff by ROC analysis was established to be on day +14 (D14) with ALC > 0.3 × 10<sup>9</sup>/L and was subsequently defined as early lymphocyte recovery (ERL). Pts with ALC ≤ 0.3 × 10<sup>9</sup>/L were deemed to have delayed lymphocyte recovery (DLR). Patients were subsequently stratified accordingly and patient, disease and transplant related factors were well balanced between the groups. Median follow up of the entire cohort was 17 (2–64.8) months. Graft characteristics: ROC analysis established optimal cellular cutoff, if present to predict ELR. Pts in the ELR group were more likely to receive CD 34 × 10<sup>6</sup>/kg < 6 (0.018), CD3 > 24 × 10<sup>7</sup>/kg (0.017) and ALC > 1.3 × 10<sup>8</sup>/kg (*P* = 0.015). We did not find a significant threshold for other allograft variables i.e. (TNC, MNC or AMC). Post HCT Outcomes: At 2 years, corresponding cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) was 16.9% vs 46.9% (*P* = 0.025) and 23.2% vs 14.2% (*P* = 0.51), for ELR and DLR cohorts, respectively. There was a trend towards improved progression free survival (PFS) and overall survival (OS) in favor of ELR vs DLR at 61.9% vs 40.1% (*P* = 0.09) and 70.1% vs 53.9% (*P* = 0.12), respectively. Median time to neutrophil and platelet engraftment was 17 and 24 days, respectively for both groups. Incidence of acute graft vs host disease (aGVHD) was similar (*P* = 0.4); however, chronic GVHD (cGVHD) was more prevalent in the ELR group at 70% vs 27%, respectively (*P* = 0.0006). On

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multivariable analysis for relapse, ELR retained its prognostic significance with HR 0.27 (0.05–0.94;  $P=0.038$ ). cGVHD and first complete remission (CR1) at the time of HCT were also protective factors from relapse in multivariable analysis. We observed that ELR is an independent predictor for relapse in patients receiving allogeneic HCT for acute leukemia with a trend towards improved OS. This is possibly related to higher incidence of cGVHD. ELR was influenced by infused allograft characteristics particularly CD34 count. Given the sample size and retrospective nature of the analysis, these important observations should be examined prospectively.

**Disclosure of conflict of interest:** None.

#### P435

##### **Influence of mixed chimerism to outcome of allogeneic stem cell transplantation (alloSCT) in patients with non-malignant diseases**

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AlloSCT is the only curative option for the treatment of hematological disorders with depression of hematopoiesis and primary immunodeficiencies. Non-myeloablative conditioning (MAC) regimens lead to long persistence of mixed chimerism (MC) in the majority of patients. Purpose: To estimate the relationship between type of hematopoietic chimerism and appearance of GVHD in patients with non-malignant diseases after alloSCT. Eleven patients (8 boys and 3 girls) with median age of 9 years (range 3–17) were included in the current study. Among them there were 7 patients with severe aplastic anemia (SAA), 2 with Fanconi anemia (FA), 1 with thalassemia, 1 with Nijmegen syndrome, treated in our Center from 2008

to 2016. Donors' sources were as follows: siblings in 7 cases, MUD (10/10) in 4 ones. In 5 cases bone marrow aspirate were used, in 6 mobilized peripheral blood hematopoietic stem cells. Conditioning regimens included fludarabine, cyclophosphamide and horse ATG for SAA patients, in FA and Nijmegen syndrome patients this scheme was augmented by low-dose busulfan. In thalassemia patient we used MAC with busulfan, fludarabine and horse ATG. In majority of cases GVHD prophylaxis consisted of tacrolimus and methotrexate combination. When alloSCT was performed form MUD patients were additionally administered with mycophenolate mofetil. Median of follow-up period was 32 mo (range 7–93). Quantitative evaluation of chimerism was done by multiplex amplification of STR loci with subsequent fragment analysis using «CORDIS Plus» kit («Gordiz LLC», Russia). We analyzed whole bone marrow and peripheral blood together with CD3+ and CD19+ lymphocyte subpopulations. Presence of ≥ 99% donors' hematopoietic cells was considered as complete donor chimerism (CC), less than 99% was considered as MC. All patients engrafted in time and all of them are alive at the time of current analysis. There were no severe life-threatening complications, infections or graft rejections. Only 2 patients achieved CC at day +28. At day +100 only these 2 patients stayed in CC. At this time point MC was mainly revealed in CD3+ lymphocytes. In 1 year after HSCT proportion of CC patients enlarged to 82% (2 patients did not achieved this time point). There is no any correlation between time of engraftment and chimerism value at day +28, either between the dose of transplanted CD34+ cells and chimerism level ( $P>0.05$  in both cases). Severe GVHD was noted only in 2 female patients with CC at day +28. In the first case it was acute GVHD grade III after HSCT from MUD, in the second case extensive form of chronic GVHD in 1 year after HSCT from sibling was observed. There are no other cases of grade III–IV acute GVHD in the observed cohort of patients. Localized form of chronic GVHD

was revealed in 4 (36%) patients. In other patients there were no signs of chronic GVHD. Despite limited number of observations we assumed that fast achievement of CC corresponds to severe GVHD. And vice versa, long persistence of MC prevented emergence of GVHD. However our findings need to be confirmed in a larger group of patients and preferably in a multicenter setting.

**Disclosure of conflict of interest:** None.

#### P436

##### **Interest of quantitative assessment of hematopoietic chimerism by real-time quantitative polymerase chain reaction after hematopoietic cell transplantation for hematological malignancies: a retrospective analysis on 347 adult patients from Rennes University Hospital**

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Chimerism (percentage of recipient versus donor-derived blood cells) is used to document engraftment after hematopoietic stem cells transplantation (HSCT). Detection of persistent host cells,<sup>1-2</sup> as well as an increase in recipient cells chimerism has been associated with impaired DFS and OS.<sup>3</sup> Quantitative real time PCR (qRT-PCR)<sup>4</sup> is a highly sensitive, reproducible method, which can detect very low levels of recipient cells. The aim of this study was to evaluate the prognostic impact of early chimerism 30 days (D30) and 100 days (D100) after HSCT and the meaning of detection of an increase of chimerism, even at low levels, during follow-up. 347 adult patients who underwent HSCT in Rennes between 2002 and 2013 were included in this retrospective study. All chimerism analyses were done with qRT-PCR using whole blood sample. Complete chimerism (CC) was defined by less than 0.1% recipient cells detected. With a median follow-up was 653 days, 101 patients relapsed with a median time of 116.5 days after HSCT. Both D30 and D100 mixed chimerism (MC) (>0.1 % recipient cells detected) were associated with an increased relapse risk ( $P=0.0003$  and  $P<0.00001$  respectively) compared to patients with CC in univariate analysis. However, when looking at subgroups analysis, D30 and D100 MC vs CC was significantly associated with increased relapse risk in this cohort for myeloid diseases ( $P=0.0049$  and  $P<0.0001$ ) but not for lymphoid diseases ( $P=0.506$  and  $P=0.059$ ). No difference in OS was observed ( $P=0.32$  and  $P=0.34$ ). More important, detection of an increased of MC (IMC) was associated with an increased relapse risk in univariate and multivariate analysis (OR=9.69 [5.42; 17.34], OR=10.05 [5.35; 18.90]), ( $P<0.0001$ ), as well as impaired OS ( $P=0.0043$ ) and DFS ( $P<0.0001$ ). Among the 103 patients with AML and at least 2 chimerism analysis available, only 3 relapsed without IMC detected but the patients' last available chimerism analysis was 75, 84 and 138 days before relapse respectively. Median levels of recipient cells detected was 1.2 %. Altogether, these results indicate that serial analyses of chimerism with qRT-PCR are a useful tool for post-transplant monitoring and might help identify patients at highest risk to relapse after transplant, especially in myeloid disease.<sup>3</sup> Monitoring frequency is critical in order to obtain the highest clinical impact, and the timing of monitoring as well as the safety and type of pre-emptive interventions still need to be explored. Considering the kinetics of the disease, frequent analysis in myeloid pathology might improve the detection of impending relapse.

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**Disclosure of conflict of interest:** None.

#### P437

##### **Molecular monitoring of engraftment kinetics and relapse by qPCR-based chimerism after unrelated hematopoietic cell transplantation**

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Host Chimerism (HC) after allogeneic hematopoietic cell transplantation (HCT) is an important parameter for measuring clinical outcome including engraftment and leukemia relapse. Although an approximately 1-log higher sensitivity of quantitative PCR (qPCR) compared to short tandem repeat (STR) has been documented in different studies, the latter remains the standard procedure for HC assessment to date. We hypothesized that qPCR could be superior to STR for monitoring the molecular kinetics of donor cell engraftment, response to donor lymphocyte infusions (DLI) and development of relapse post-HCT. We analyzed 30 patients (pts) who underwent mainly 10/10 HLA-matched unrelated HCT mostly for acute myeloid or lymphatic leukemia at the University Hospital Essen between 2006 and 2013. Transplant conditioning was mostly myeloablative and GvHD prophylaxis was by cyclosporin A and methotrexate without anti-thymocyte globulin (ATG). Median follow-up of pts was 1504 days (d) (317–2891). Cytomegalovirus (CMV) reactivation in the first 100d post-transplant was measured by pp65 ( $N=22$ ) or PCR ( $N=8$ ). A total of 459 retrospective genomic DNA samples from peripheral blood (PB;  $N=364$ ) or bone marrow ( $N=95$ ) collected between d21 and d2302 post-transplant were available for HC analysis in parallel by STR (Mentype Chimera, Biotype) and qPCR (AlleleSeq, Abbott). Threshold for HC positivity in qPCR was set at 0.1% following published protocols. Concordance in HC analysis between qPCR and STR was found in 365/459 (79.5%) samples, with all 94 discordant cases positive in qPCR but negative in STR. Engraftment could be assessed in 110 samples drawn at d21–d213 from 18 pts without relapse in the first 6 months post-HCT. These samples showed concordant negative or positive qPCR and STR results reflective of full donor engraftment or persistent mixed chimerism (PMC) in 5 and 3 pts, respectively. In 2 pts, qPCR but not STR documented delayed conversion to full donor chimerism until d200. In the remaining 8 pts, positive results in qPCR but not STR during early engraftment were observed exclusively in BM, in particular those drawn before d35 post-HCT. qPCR but not STR was also able to document the kinetics of conversion to full donor chimerism which took 126d and 196d in 2 pts receiving DLI for treatment of PMC and relapse, respectively. 8 informative relapses could be assessed in 33 samples drawn at least 192d before onset. 6/6 BM and 23/26 PB were positive in qPCR, compared to 2/6 BM and 2/23 PB in STR, with a sensitivity of 8/8 (100%) and 3/8 (37.5%) relapses, respectively. Consistent with previous reports on a protective effect of early CMV reactivation on relapse in GvHD prophylaxis regimens without ATG, relapse occurred in 1/10 (10%) pts who experienced CMV reactivation in the first 100d post-transplant, compared to 10/20 (50%) pts who did not. No apparent

associations were observed between early CMV reactivation and engraftment kinetics post-HCT. HC assessment by qPCR is highly concordant with STR, but markedly superior for molecular monitoring of engraftment kinetics and relapse. Positive qPCR results in BM should be interpreted with caution during early engraftment, while both BM and PB were highly informative for relapse in our series. These results advocate the feasibility and clinical utility of qPCR for post-HCT HC monitoring in routine use.

**Disclosure of conflict of interest:** The commercial assays for qPCR chimerism analysis were provided by Abbott Molecular free of charge.

#### P438

##### **Ponatinib post-transplant treatment in patients with high-risk Philadelphia chromosome positive leukemia**

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Tyrosine kinase inhibitor (TKi) has become the standard of care in patients (pts) with chronic myeloid leukemia (CML) and an unavoidable tool in the combined therapy for pts with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Nevertheless, allogeneic stem cell transplantation (HSCT) remains the standard therapy of ALL Ph+ and of CML pts failing 1st line therapy with TKi, with failure or insufficient response or intolerance or mutations resistant to 2nd generation TKi, or in the advanced phase at diagnosis. In the past decade the feasibility and safety of post-HSCT imatinib administration as prophylactic or therapeutic strategy was confirmed. Second and 3rd generation TKi administration after HSCT is under investigation. Here we are reporting our experience in post-HSCT treatment with the 3rd generation TKi ponatinib in 5 pts treated between 2011 and 2016 at our Institution. Pts data and information were collected from Institutional database and chapters revision. A written consent was given by pts allowing the use of medical records for research in accordance with the Declaration of Helsinki. Pts and diseases features are reported in Table 1. Pre-transplant treatment for the ALL Ph+ patient consisted of chemotherapy combined with dasatinib, followed by a 1st MUD HSCT and dasatinib in maintenance. The patient relapsed 1 year after HSCT with documentation of mutation V299L. Ponatinib was introduced as salvage treatment to bridge 2nd haplo HSCT. Pre-transplant treatment for the CML patients consisted of TKi therapy with combination of chemotherapy in case of uncontrolled progression of disease. Two pts received a 1st MUD HSCT but relapsed respectively 5 months and 4 years later. Ponatinib was introduced as salvage treatment to bridge 2nd haplo HSCT. Four pts received ponatinib 45 mg daily before the last HSCT: one patient achieved sustained major molecular response, 4 pts obtained transient response. All pts were presenting 2nd generation TKi resistant mutations (Table 1). Ponatinib was started at a median of 157 days after HSCT (range, 117-583) as salvage treatment in overt relapse (3 cases), prophylaxis (1 case) or preemptive therapy (1 case). Acute GvHD was diagnosed in 4 pts before ponatinib administration, 2 of them also experienced chronic GvHD. No new cases of GvHD were observed after initiation of ponatinib. Immunosuppressive treatment and azoles treatment were discontinued before ponatinib in all but one patient who was under combined treatment for chronic GvHD: therapeutic drug monitoring was closely performed without evidence of drug-drug interaction. Pts were regularly evaluated for toxicities and monitored for cardiovascular events.

No serious adverse events were reported in our experience: we administered ponatinib at a median maximum dosage of 30 mg daily (range, 15-45 mg), for a median of 24 weeks (range, 4-132 weeks). At last evaluation one patient maintained the status of molecularly undetectable leukemia (follow-up post HSCT: 34 months) and one major molecular response (follow-up post HSCT 29 months). Three patients who received therapeutic ponatinib in overt relapse did not respond and died for progressive disease. Ponatinib is safe and well tolerated as bridge to HSCT and to maintain disease control after transplant. Prophylaxis targeted therapy and preemptive therapy with ponatinib may lead to the reduction of disease relapse for high-risk Ph+ leukemia.

**Disclosure of conflict of interest:** None.

#### P439

##### **Rapid immune reconstitution post CD45RA depleted HSCT leads to better engraftment, reduced risk of invasive viral infections and superior graft versus leukemia effect**

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Rapid immune reconstitution following CD3/CD45RA depleted HSCT results in reduced incidence of invasive viral infections, better engraftment without loss of graft versus leukemia effects. Chetan Dhamne MD, Mariflor Villegas MD, Frances Yeap MD, Soh Teck Guan, Allen Yeoh MD, Quah Thuan Chong MD, Tan Poh Lin MD National University Hospital Singapore Introduction: Graft manipulation is an important strategy in haploidentical stem cell transplantation. At NUH Singapore we have adopted the CD45 RA depletion to ameliorate graft versus host disease. Materials and Methods: We have transplanted 14 leukemia patients with CD3 depleted HSCT followed by CD45RA depleted donor lymphocyte infusion. No additional GVHD prophylaxis or GCSF was used. Results: 100% patients achieved primary engraftment. Median time for neutrophil engraftment (> 500/ $\mu$ L without GCSF) was 14 days (range 7-17 days), platelet engraftment (> 50 000) was 13 days (range 10 to 29 days). Immune reconstitution was rapid with median CD4 and CD8 cell counts > 200/ $\mu$ L at day 30. By day 200 median CD4 count was 390/ $\mu$ L (range 312-817/ $\mu$ L). No patient developed grade IV acute GVHD. There was a significantly reduced incidence of invasive viral infections as compared to conventional transplants. Importantly, all patients achieved complete remission (CR) on day +21 and remained in CR for longer time as compared to conventional transplants. **Conclusion:** Our preliminary data suggests that rapid immune-reconstitution of NK cells and T cells with this strategy correlates with reduced infection related mortality without loss of graft versus leukemia effects.

**Disclosure of conflict of interest:** None.

#### P440

##### **Reconstitution of cellular immunity after T-cell replete haploidentical stem cell transplantation by use of post-transplant cyclophosphamide**

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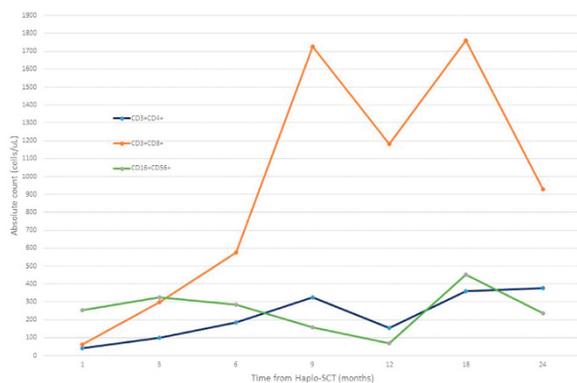
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The use of post-transplantation high-dose cyclophosphamide (PT-CY) has overcome the need for extensive depletion of T lymphocytes from haploidentical donor grafts, which

traditionally resulted in severe and prolonged immunosuppression. It is therefore relevant to investigate the degree and the tempo of immune reconstitution after T cell replete haploidentical stem cell transplantation (haplo-SCT) by use of PT-CY. We prospectively monitored cellular immunity in 15 consecutive adult patients (male/female: 9/6), who underwent haplo-SCT with PT-CY for myeloid ( $n=12$ ) or lymphoid ( $n=3$ ) malignancies. The median age at transplant was 56 (range, 27–68) years. The conditioning regimen was myeloablative in 10, reduced-intensity in 4, and non-myeloablative in 1 case. The source of the graft was peripheral blood ( $n=7$ ) or bone marrow ( $n=8$ ). In addition to PT-CY, graft-versus-host disease (GvHD) prophylaxis included tacrolimus and mycophenolate mofetil. Absolute counts of CD3+CD4+, CD3+CD8+, CD19+ and CD16+CD56+ cells were measured by flow cytometry at 1, 3, 6, 9, 12, 18 and 24 months post transplant. The median doses of infused CD34+ and CD3+ cells were 4.13 (range, 1.16–9.61)  $\times 10^6$ /Kg and 4.45 (range, 2.2–38)  $\times 10^7$ /Kg, respectively. Neutrophil engraftment ( $>500$ /uL) was achieved at a median of 18 (range, 16–35) days, whereas platelet engraftment ( $>20\,000$ /uL) was observed at a median of 23 (range, 13–54) days. Seven patients developed acute GvHD (grade I/II: 6, grade III: 1). Chronic GvHD occurred in 3 patients, and was extensive in the 2 of them. Cytomegalovirus infection was detected in 9/15 cases at a median interval of 42 (range, 30–89) days post transplant. Two patients were administered rituximab for Epstein–Barr virus reactivation at 10 months, whereas one patient developed BK virus-associated hemorrhagic cystitis at 2.5 months following haplo-SCT. There was 1 death due to GvHD and infection at 7 months post transplant. At a median follow-up of 12 (range, 3–33) months, 14/15 patients remain alive and disease-free. The absolute counts of T and B cells were extremely low early post transplant, while NK cells recovered from the first month (mean count, 254/ $\mu$ L). The number of CD8+ T cells started to increase beyond the first month, and exceeded lower normal limit at 3 months (mean count, 296/ $\mu$ L). CD4+ T cells remained in general low ( $<100$ / $\mu$ L) for the first 3 months, increased moderately by 6 months (mean count, 186/ $\mu$ L), and approached lower normal values at 9 months (mean count, 325/ $\mu$ L) [Figure 1]. Of note, CD4+CD45RA+ naïve T cells remained significantly impaired (absolute count range, 4–52/ $\mu$ L) in all 7 patients in which they were assessed beyond the 1st year from transplant. CD19+ B cells were suppressed for the entire first trimester (mean count at 3 months, 68/ $\mu$ L), but increased rapidly between 6 and 9 months (mean counts, 165/ $\mu$ L and 366/ $\mu$ L, respectively). In haplo-SCT with PT-CY, reconstitution of cellular immunity can be achieved at adequate levels by 6–9 months following transplant. The observed deficit in the recovery of naïve T-helper cells may be related to a possible effect of PT-CY on thymopoiesis and warrants further investigation.

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**Figure 1.** Reconstitution of T cell Subsets (CD4+ and CD8+) and NK Cells (CD16+CD56+) After Haplo-SCT with Post-Transplant Cyclophosphamide (PT-CY)



**Disclosure of conflict of interest:** None.

#### P441

#### Stable mixed chimerism after hematopoietic stem cell transplantation in patients with sickle cell disease

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Hematopoietic Stem Cell Transplantation (HSCT) is a curative therapy for patients with Sickle Cell Disease (SCD). Hemoglobin A in SCD ameliorates the manifestations of the disease and this could be achieved with stable mixed chimerism after a reduced intensity HSCT. This study aims to estimate the proportion of patients who develop mixed chimerism after HSCT for SCD and to characterize its progression in patients who develop it. This is a retrospective cohort study conducted at Sultan Qaboos University Hospital (SQUH) bone marrow transplant unit in Oman. We included all patients with SCD who received HSCT over the course of 10 years between May 2007 to May 2016. Patients who received second HSCT were excluded. Short tandem repeat polymerase chain reaction was used for chimerism assessment. Mixed chimerism was defined as 5–95% chimerism at 6 months from HSCT. The data was analyzed by R program 3.1.2.  $\chi^2$  or Student *t*-test were used to assess the impact of acute graft versus host disease (aGvHD) prophylaxis, age at transplantation, gender, red blood cell antigen alloimmunization, preparative regimen, and ferritin on the development of mixed chimerism. We included 56 eligible patients. The median follow-up time after HSCT was 26 months (Interquartile Range: 17.3–50.3 months). The mean age at transplant was 19.9 years (Standard deviation: 8.44). Fifty-nine percent of patients were male. Most patients had S/S genotype (77%), followed by S/beta-thalassemia mutation (20%). The indications for BMT were: stroke in 7%, acute chest syndrome (ACS) in 9%, recurrent vaso-occlusive crisis (VOC) in 38%, stroke and ACS in 7%, ACS and VOC in 31%, orbital compression syndrome in 2%, stroke and Moyamoya disease in 4%, and Moyamoya disease in 2%. The two most frequently used preparative regimens were Busulfan/Fludarabine/ATG in 49% and Thiotepa/Treosulfan/Fludarabine in 42%. Twenty-five percent of patients developed mixed chimerism at six months after HSCT. On follow up of patients with mixed chimerism, 10% rejected the graft, 20% developed complete chimerism, and 70% continued to be in mixed chimerism. Preparative regimen and the development of aGvHD were statistically significant predictors of mixed chimerism at 6 months (*P* values: 0.00079 and 0.01817 respectively). Age at transplant, gender, red blood cell antigen alloimmunization, and ferritin were not statistically significant predictors of the mixed chimerism (*P* > 0.05). The study confirmed that mixed chimerism can commonly be achieved in patients with SCD after HSCT and in majority, it remains stable on long-term follow-up. Reduced intensity preparative regimen and lack of aGvHD predicts the development of mixed chimerism. Larger prospective studies are needed to confirm these results.

**Disclosure of conflict of interest:** None.

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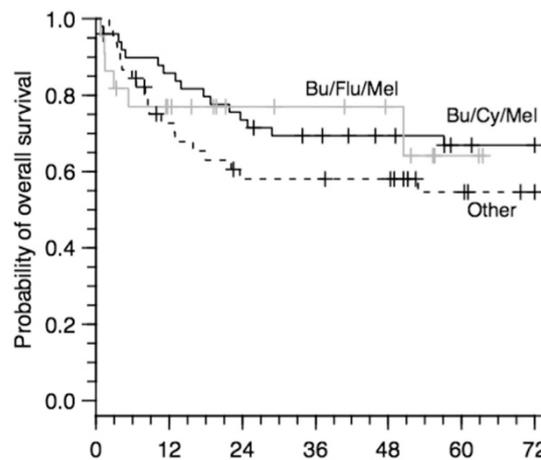
**Allogeneic hematopoietic stem cell transplantation for juvenile myelomonocytic leukemia in France: a retrospective study of Société Française De Greffe De Moelle Et De Thérapie Cellulaire**

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Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive childhood haematological malignant disorder. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only proven curative therapy. Despite strong myelo-ablative conditioning regimen, JMML still remain associated to engraftment failure and relapse. This study reports more than 25 years (1986–2015) of French experience in HSCT for children with JMML. The outcome of 117 children transplanted between March 1986 and October 2015 in 23 French centres was retrospectively studied. Overall, 136 allografts were performed during these period. The published EWOG-MDS criteria have been used to make the diagnosis of JMML and checked retrospectively for those patients transplanted before 1989. The median follow-up from transplant was 78 months (1–219). In this work, we compared the results of the preparative regimen recommended by the EWOG-MDS/EBMT trial, which is an association of three alkylating agents: busulfan, cyclophosphamide and melphalan (Bu/Cy/Mel) with the conditioning regimen busulfan, fludarabine and melphalan (Bu/Flu/Mel) established by the Japanese group. The median age at diagnosis was 1.4 years (range, 0.0–15.7 years). There was a majority of male patients (69%). The median HbF level was 15% (0–77), median monocyte count was 5.109/l (range: 0–74.8), median platelet count was 64.109/l (4–377), median marrow blasts was 5% (0–37). Above 96 patients who were explored by marrow karyotype, 27% of them had a monosomy 7. Mutations in PTPN11 were detected in 33 patients. Fifty patients (43%) were treated with the Bu/Cy/Mel regimen, whereas 22 patients (19%) received the Bu/Flu/Mel regimen. At 6 years, the overall survival (OS) was 62% (95% CI: 53–72). Nineteen and 9 patients developed VOD after Bu/Cy/Mel and Bu/Flu/Mel conditioning regimen, respectively. The cumulative incidence of aGVHD 3–4 was 26% (95% CI: 19–35). The 6-year cumulative incidence of relapse and non-relapse mortality was 30% (95% CI: 22–39) and 18% (95% CI: 11–25), respectively. The median delay of relapse was 90 days (range 15–1330). Among relapsing patients, 16 were transplanted twice and one underwent 3 HSCT. In multivariate analysis, female donor to male recipient sex-mismatch, CMV status, total body irradiation and RAS-double mutation/other additional mutation predicted poorer outcomes. The Bu/Flu/Mel conditioning regimen was associated with a decreased risk of relapse. However, there was no statistical difference for OS between the two main preparative regimens, Bu/Cy/Mel vs Bu/Flu/Mel. Our results show that allogeneic HSCT may cure approximately 60% of patients with JMML and are similar to the best results published by other groups. Relapse represents the main cause of treatment failure and a second HSCT should be proposed. Despite a decreased risk of relapse with the Bu/Flu/Mel regimen, there was no statistical difference in terms of OS between the two main conditioning regimens, Bu/Flu/Mel vs Bu/Cy/Mel.

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No. at risk:	Months after transplant						
	0	12	24	36	48	60	72
Bu/Cy/Mel	50	42	36	32	29	25	24
Bu/Flu/Mel	22	14	9	8	6	2	0
Other	45	30	23	23	22	16	13

Disclosure of conflict of interest: None.

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**Allogeneic hematopoietic stem cell transplantation in patients with pediatric myelodysplastic syndrome and myeloproliferative neoplasms: a single center experience from Turkey**

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Allogeneic hematopoietic stem cell transplantation (AHSCT) is the only curative treatment modality for the majority of pediatric patients with myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN). The purpose of this study is to evaluate overall (OS) and failure (relapse or death from any cause) free survivals (FFS), non-relapse mortality (NRM) and relapse incidence in children who underwent AHSCT for MDS or MPN in a single center from Turkey. We retrospectively analyzed 45 AHSCT carried out in 43 patients (median age: 9.2 years; range: 0.4–18; 24 males). Thirty four had primary MDS and 9 had secondary MDS. According to the modified WHO MDS and MPN classification, 18 had refractory cytopenia (RC), 12, refractory anemia with excess blast (RAEB), 1, refractory anemia with excess blast in transformation (RAEB-t) and 12, juvenile myelomonocytic leukemia (JMML). Amongst patients with secondary MDS, 4 had been treated for acute myeloid leukemia, 2 had been treated for non-Hodgkin's lymphoma and 1 had been treated for acute lymphoblastic leukemia, retinoblastoma and osteosarcoma, each, previously. Donors were related in 18 transplantation (5 haploidentical transplantation) and the stem cell resources were bone marrow ( $n=27$ ), peripheral blood ( $n=14$ ), cord blood ( $n=2$ ) and bone marrow +peripheral blood ( $n=2$ ). Three-year FFS and OS for patients with MDS were 55% and 57.0%, respectively; and for patients with JMML, 50% and 64%, respectively. Crude incidence of NRM and relapse for entire group were 33% and 22%, respectively. AHSCT offers durable FFS and OS for a significant group of pediatric patients with MDS and MPN. Less toxic

conditioning regimens could result in better results in some patients.

**Disclosure of conflict of interest:** None.

#### P444

##### **Allogeneic stem cell transplantation in children with autism**

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Autism Spectrum Disorders (ASD) are severe heterogeneous neurodevelopmental abnormalities characterized by dysfunctions in social interactions and communication skills, restricted interests, repetitive, and stereotypic verbal and non-verbal behaviors. The etiology of ASD remains unknown, but recent studies suggest a possible association with altered immune responses and ASD. Inflammation in the brain and central nervous system has been reported with microglia activation and increased cytokine production in postmortem brain specimens of individuals with ASD. Other studies have established a correlation between ASD and family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. The paracrine, secretome, and immunomodulatory effects of stem cells would appear to be the likely mechanisms of application for ASD therapeutics. We describe two cases of patients with ASD who underwent HSCT for acute lymphoblastic leukemia (ALL) and whose symptoms were markedly decreased like an improvement of social interaction, communication, and behaviors. The first patient is an 11-year-old girl with ASD who was diagnosis with Ph-positive ALL in October 2011 (at the end of treatment, BCR-ABL remained positive). She underwent a matched sibling HSCT in March 2015. The conditioning regimen was total body irradiation (TBI) and cyclophosphamide. During the 20-month follow-up period, we observed improvement in social interaction, communication, and behaviors (according to The Childhood Autism Rating Scale-CARS). The second case is a 7-year-old boy with ASD, Asperger Syndrome, who was diagnosis with ALL in September 2012. He presented with bone marrow and testicular relapse in May 2015 and underwent a matched unrelated HSCT in November 2015. The conditioning regimen used was Etoposide, ATG and TBI. During the 12-month follow-up period, we observed improvement in social interaction, communication, and behaviors (according to CARS). There is no treatment for ASD thus every effort to minimize the symptoms are valuable. In both cases, social interaction was significantly increased, and the aggressive behaviors decreased. Clinical cases have reported responses in autistic children receiving cord blood CD34+ cells. Several incurable neurological disorders have shown benefits with cellular therapy. Thus, autism should be explored as an indication. Clinical studies are an immediate need to fully explore its potential in autism.

**Disclosure of conflict of interest:** None.

#### P445

##### **Association between conditioning regimen intensity and nutritional aspects in allogeneic hematopoietic stem cell transplantation in children and adolescents**

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Conditioning is the initial phase of hematopoietic stem cell transplantation, based on high dose chemotherapy, combined or not with total body irradiation, aiming to eradicate the disease and prepare the environment of the bone marrow for

the new cells. Conditioning regimens can be characterized as myeloablative or non-myeloablative. During the period of conditioning and immunological reconstitution, signs and symptoms of the gastrointestinal tract are frequent, negatively influencing oral food intake, and may require the use of complementary nutritional therapies, aiming at an adequate caloric intake with the objective of avoiding decreasing in the nutritional status. The study aims to describe the association between the regimen intensity and the nutritional aspects during hospitalization of children and adolescents undergoing allogeneic hematopoietic stem cell transplantation (HSCT) at a tertiary hospital. A retrospective study with medical records of patients undergoing allogeneic HSCT, aged between 0 and 19 years of age (incomplete) between January 2009 and December 2014. Data were collected (regimen intensity, clinical signs of mucositis and nutritional therapies used) during the hospitalization and analyzed by the relative risk (RR). Sixty-three patients were evaluated, being 56% male, with a median age of 10 years. Nineteen types of conditioning protocols were used. Of these, 64% were high intensive regimen and 36% were low intensive regimen. The four most applied (59% of cases) were BuCy (Busulfan + Cyclophosphamide) with and without ATG (thymoglobulin), as well as CyTB (Cyclophosphamide+total body irradiation), also with and without ATG. Mucositis were observed in 83% of patients, being 50% grade 3 and grade 4. The association was positive when analyzed the regimen intensity (myeloablative) with mucositis (RR=1.51 (1.10–2.08)) as well with the use of parenteral nutrition (RR=2.49 (1.17–5.13)). Patients showed high prevalence of mucositis during hospitalization decreasing food intake, being necessary to use the parenteral nutrition. Myeloablative regimen needed more nutritional therapy intervention when compared to non-mioloablative regimen. Results demonstrate that an appropriate nutritional screening tool considering these aspects could help to intervene earlier maintaining an adequate nutritional status.

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**Disclosure of conflict of interest:** None.

#### P446

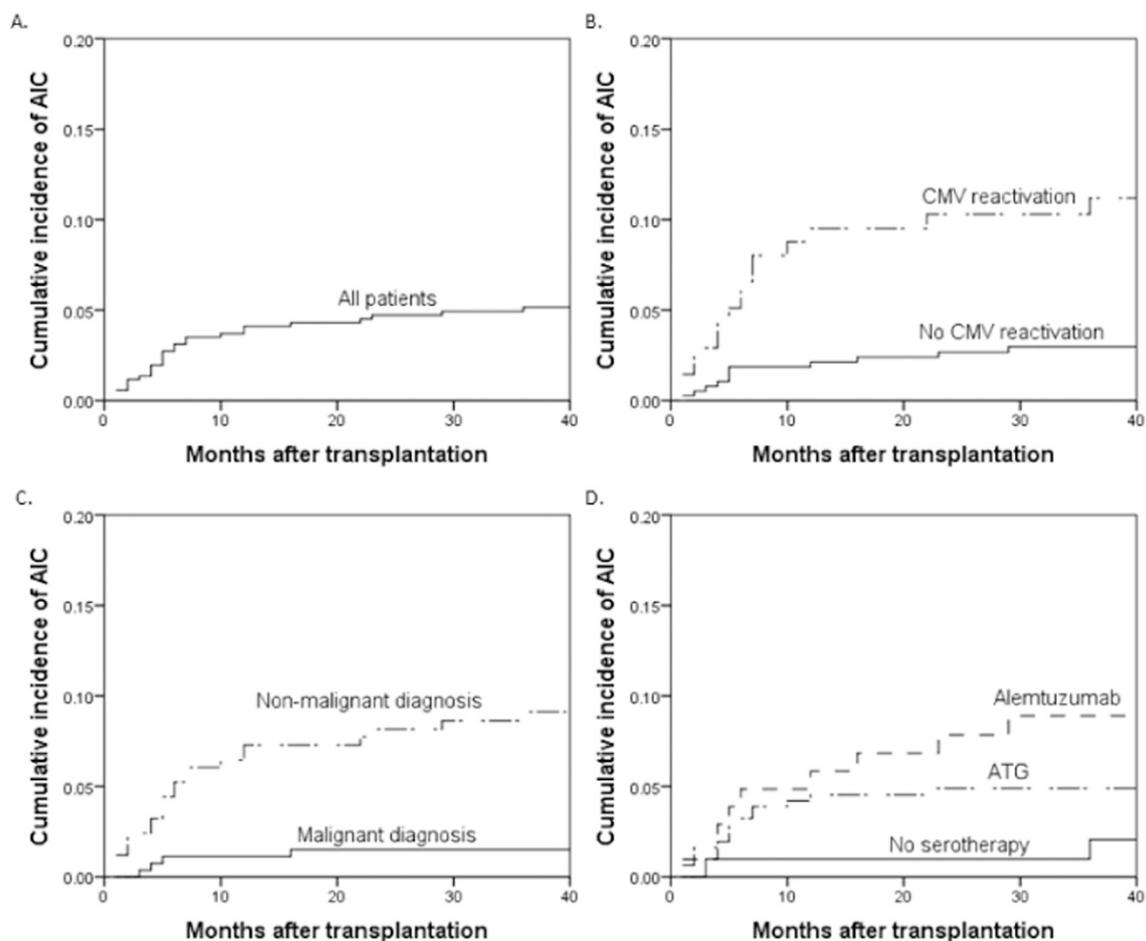
##### **Autoimmune cytopenia following allogeneic hematopoietic stem cell transplantation in pediatric patients**

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Autoimmune cytopenia (AIC) is a potentially serious complication of hematopoietic stem cell transplantation (SCT). Autoimmune hemolytic anemia (AIHA) is the most common AIC, followed by immune thrombocytopenic purpura and autoimmune neutropenia. AIC after SCT is considered difficult to treat and associated with high morbidity and mortality. The aim of this cohort study is to evaluate incidence, outcome, potential risk factors and current treatment strategies and to explore the immune dysregulation predisposing to AIC. The EBMT-Promise database was accessed to identify all pediatric SCTs between 2000 and 2016 complicated by AIC at our

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center. Potential risk factors (i.e., age, gender, diagnosis, donor type, stem cell source, conditioning regimen) for AIC after SCT were assessed using univariate and multivariate Cox regression analysis. In addition, we summarized treatment decisions of all AIC patients. A nested matched case-control study was performed to search for possible biomarkers for AIC. Of 531 consecutive SCTs, 27 were complicated by the development of AIC (cumulative incidence 5.2%) at a median of 5 months post-SCT (Figure). AIHA was the most common AIC (48%), followed by combinations of two or more AICs (Evans syndrome, 33%). Non-malignant disease, young age, alemtuzumab serotherapy pre-SCT, non-TBI based conditioning regimen and CMV reactivation were associated with AIC in univariate analyses. Using multivariate Cox regression analysis, non-malignant disease (HR 3.6,  $P=0.028$ ), alemtuzumab use (HR 2.4,  $P=0.035$ ) and CMV reactivation (HR 3.7,  $P=0.013$ ) were independently associated with AIC (Figure). For patients with CMV reactivation, diagnosis of AIC was made at a median of 4 months (IQR [1–8]) after detection of maximum viral load. In our nested case-control analysis, serum levels of individual anti- and pro-inflammatory, and regulatory cytokines did not differ significantly between patients and controls. However, the cytokine profile of AIC patients appeared to skew towards a more pronounced Th2 response, compared to controls. First-line treatment, usually with prednisone and/or IVIG, or a wait-and-see approach led to resolution of AIC in 9 (33%) cases. Second and subsequent-line therapies, often in combination with continuation of other treatments, consisted of rituximab ( $n=16$ ), bortezomib ( $n=7$ ) or sirolimus ( $n=3$ ) and eventually led to resolution of AIC in 44%, 57% and 100% of cases,

respectively. Overall survival of AIC patients was 78%. In this retrospective cohort study, we identified CMV reactivation post-SCT, alemtuzumab use and non-malignant disease as independently associated clinical risk factors for the development of AIC. Treatment with first-line therapy was mostly insufficient. For patients with severe AIC, rituximab, bortezomib or sirolimus can be regarded as promising step-up therapies. Figure legend: Cumulative incidence of the development of autoimmune cytopenia after SCT. (A) All patients. (B) Patients with CMV reactivation versus patient without CMV reactivation. (C) Patients with malignant disease versus patients with non-malignant disease. (D) Patients who received alemtuzumab versus patients who received ATG versus patients who received no serotherapy.

**Disclosure of conflict of interest:** None.

#### P447

#### BK polyoma virus disease after allogeneic hematopoietic stem cell transplantation: the experience of single center

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BK polyoma virus infection is an evolving challenge following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Several risk factors have been reported including BU-containing myeloablative conditioning, unrelated donors and GVHD. In contrast to early -onset HC, late-onset HC mainly results from polyomavirus BK and less frequently adenovirus or cytomegalovirus. Reactivation of human polyomavirus BK

(BKV) may cause polyomavirus-associated nephropathy or polyoma virus-associated hemorrhagic cystitis in bone marrow-transplant patients. We present 19 patients with BK polyoma virus (BKV) associated hemorrhagic cystitis and 2 patients with BK polyoma virus associated hemorrhagic cystitis and nephritis. Between 2013 and 2016, 124 patients received an allogeneic BMT at Acibadem Adana Hospital Pediatric Bone Marrow Transplantation Unit. 21 patients occurred BKV associated hemorrhagic cystitis and nephritis. BKV was detected in the urine analysis and blood by PCR (polymerase chain reaction) in all patients. We presented 21 patients with BKV infection, age ranging from 3 to 20 with an average of 13.1 years. They underwent allogeneic BMT due to thalassemia major (13 patients), aplastic anemia (4 patients) and acute lymphoblastic leukemia (4 patients). The patients were treated with hydration, continuous bladder irrigation, ciprofloxacin, and weekly intravesical hyaluronic acid instillation for four weeks, and cidofovir. Fourteen patients showed complete resolution of hematuria. One patient with refractory above these therapy also received hyperbaric oxygen and recombinant factor VIIa (rFVIIa, NovoSeven; Novo Nordisk, Bagsvaerd, Denmark). Hemodialysis was performed in two patients who developed renal failure due to nephritis. BKV is ubiquitously present in the general population.<sup>1</sup> Reactivation of infection occurs under conditions of immunosuppression, particularly HSCT or renal transplantation, and causes late-onset HC. BKV The management of BKV cystitis and nephritis sometimes may be very difficult and refractory all treatments, we presented our experience of BKV infection and management in transplanted patients in our center.

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**Disclosure of conflict of interest:** None.

**P448**

**Breakthrough invasive fungal disease in high-risk hematologic malignancies in children: a single-center experience**

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Patients with high-risk hematologic malignancies (HRHM) are among those in the highest risk group for developing invasive fungal disease (IFD), especially mold infections. Allogeneic hematopoietic stem cell transplantation (AL SCT), acute myeloid leukemia (AML), refractory and relapsed acute lymphoblastic leukemia (ALL), Myelodysplastic Syndromes and chronic extensive graft-versus-host disease are considered HRHM. IFD are a major cause of morbidity and mortality in these patients, however, the optimal strategy for antifungal

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**Table 1**

Patient	1	2	3	4
Gender	Female	Male	Female	Male
Age at diagnosis of IFD (years)	11	5	12	15
Diagnostic Status at IFD	Refractory ALL	High Risk ALL	Refractory AML	Secondary AML
Treatment	SHOP-2005 ALL Allogeneic SCT VP-CY-TB	SHOP-2005 ALL Autologous SCT BuCyTT SEHOP-2008	SHOP-2007 AML Clofarabine + citarabine	SHOP-2007
IFD Diagnosis Criteria according EORTC/MSG	Proven Scedosporium	Probable Aspergillus	Proven Aspergillus	Probable Aspergillus
IFD treatment	Combined therapy (Voriconazole + Ambisome®)	Combined therapy (Voriconazole + Ambisome® + Aerosolized Abelcet®)	Combined therapy (Voriconazole + Ambisome® + Caspofungin + Aerosolized Abelcet®)	Combined therapy (Voriconazole + Caspofungin) (allergy to Ambisome®)
IFD follow-up	Progression	Cured	Progression	Cured
Current status	Death	Death	Death	Alive
Cause of death	IFD	Disease progression	Disease progression + IFD	-

prophylaxis in this population is not well defined yet. We performed a retrospective, observational study to investigate documented IFD during antifungal prophylaxis in children with HRHM who were admitted in our Unit between 2010 and 2016. Demographic and clinical data were collected from patient's electronic medical records. All patients were treated with prophylactic voriconazole (VCZ) according to our local practice. Oral administration was preferred when available. VCZ therapeutic drug monitoring (TDM) was not available in our center until June 2016. Breakthrough IFD was defined as occurrence of a proven or probable IFD according to EORTC/MSG criteria while on VCZ prophylaxis ( $\geq 7$  days of treatment) or within 15 days after discontinuation of prophylaxis. During the study period, 75 HRHM patients were treated with prophylactic VCZ in our unit. 4 patients out of 75 developed a breakthrough IFD. Patient's demographic characteristics, main diagnosis and treatment are collected in Table 1. Initial and maintenance VCZ doses are adjusted by weight in all patients except in patient-4 (adjusted according to VCZ plasma level). Adherence and tolerance to treatment was excellent in all patients.

**Disclosure of conflict of interest:** None.

#### P449

### Chimerism evaluation after allogeneic haematopoietic stem cell transplantation in children with non malignant diseases.

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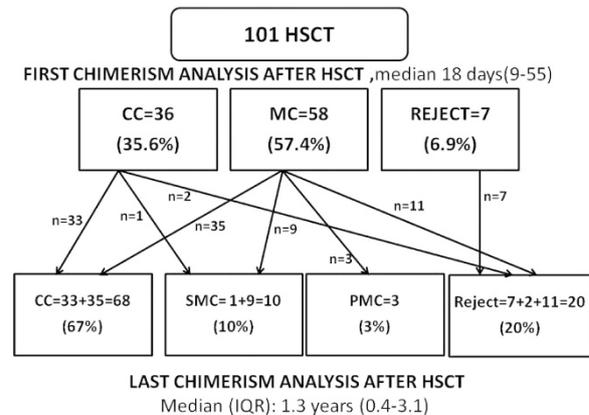
DNA analysis with short tandem repeat (STR) is the gold standard to monitor chimerism after allogeneic haematopoietic stem cell transplantation (allo-HSCT).<sup>1</sup> The aim of this study was to analyze STR based chimerism in children with non malignant disease and to describes its evolution at different time points after HSCT. All transplants performed in our institution from January 2004 to March 2016 in children with non malignant diseases were included in the study. Chimerism was evaluated with STR in at least t three time points after HSCT. On the basis of percentage of recipient-donor cells the chimerism was classified as (1) complete chimerism (CC) if autologous cells (AC) were  $< 1\%$  and; (2) mixed chimerism (MC) if AC was  $\geq 1\%$ . Over time variations were classified as (3) stable mixed chimerism (SMC) when fluctuations of AC were  $< 5\%$ ; and (4) mixed progressive chimerism (PMC) when AC were  $\geq 15\%$ .<sup>2-3</sup> 101 HSCTs performed in 85 patients (pts) were included: 72 children with a median age of 2.01 yrs (IQR 0.62–7.35 yrs) at diagnosis and 6.2 yrs (IQR 2–11 yrs) at HSCT received one HSCT (84.7%), 10 pts two HSCT (11.8%), and 3 pts three HSCTs. Primary diagnosis were bone marrow failures in 37 pts (43.5%), primary immunodeficiencies in 25 (29.4%), inborn errors of metabolism in 15 (17.5%) and haemoglobinopathies in 8 (9.41%). The donor was match related in 23 (23%) procedures, match unrelated in 63 (62%), and haploidentical in 15 (15%); stem cell source was bone marrow in 55 (54%), peripheral blood in (26%) and cord blood in 20 (20%). Conditioning regimen (CR) included Busulfan in 19 HSCTs (18.8%), Treosulfan in 33 (32.7%), while 48 HSCTs (47.5%) were conditioned with reduced intensity CRs (including low dose of TBI in 9); 1 pt did not received CR. GvHD prophylaxis was based on CSA/MTX (or MMF) in association with ATG (69) or Alemtuzumab (16); recipients of TCR $\alpha\beta$ /CD19 depleted haploidentical graft did not received post transplant immunosuppression. Engraftment was observed in 87 HSCTs (79 after 1<sup>st</sup>, 7 after 2<sup>nd</sup> and 1 after 3<sup>rd</sup> HSCT) after a median of 18 day (IQR 14–23 days). Acute GvHD occurred in 45 HSCTs at risk (52%), and it was severe (gr. III–IV) in 20 (23%), chronic GvHD in 31 (31%). At last follow-up (median 4.35 yrs), 75 (88%) pts were

alive, while 10 pts are dead for infections ( $n=5$ ), VOD ( $n=1$ ), c-GVHD ( $n=3$ ) and vascular event ( $n=1$ ). Figure 1 reported the evolution of chimerism over time. In our experience in children with non malignant disease, CC increased from 36% to 67% at subsequent analyses. 60% of pts with MC at 1<sup>st</sup> evaluation became CC, 16% remained SMC, 5% evolved in PMC, and 19% rejected. Only 2 pts with CC at first time point rejected the graft. This study highlight the extreme variability of chimerism in the early post transplant course of children with non malignant disease and confirmed the relevance of performing serial analysis to monitor and, if necessary, improve graft function.

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[P449]



**Disclosure of conflict of interest:** None.

#### P450

### Clinical outcome, safety and rapid immunologic reconstitution in C45RA-depleted haploidentical transplantation in paediatric acute leukemia treatment

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Allogeneic hematopoietic stem-cell transplantation (HSCT) is the therapeutic treatment for different malignant conditions. Haploidentical HSCT expands this treatment to 40% of patients without proper matched-donor. Haplo-HSCT in the paediatric setting requires T-cell depletion, which carries profound immunosuppression and high risk of graft failure. Naive T-cells identified by CD45RA expression are believed to cause graft-versus-host-disease (GvHD), while CD45RA- T-cells are memory cells that provide anti-infection and anti-tumoral effects. Depleting CD45RA+ naive cells and retaining memory T-cells in the graft is a novel approach to haploidentical HSCT for children. 18 children with high risk leukemia (6 AML, 12 ALL) received CD45RA-depleted haploidentical HSCT following non-myeloablative conditioning. Cell-selection performed on G-CSF-mobilized peripheral blood. Two cellular products obtained using CliniMACS device, infused to each patient: a CD34 selection and a CD45RA depletion from the CD34-negative fraction. Product infused contained a median of 6.04 (range 4.04–9.93)  $\times 10^6$ /Kg CD34+ cells and a median of 6.5 (range 1.3–490)  $\times 10^3$ /Kg of CD3+ cells in the CD34-selected

graft. The second product was the CD45RA depletion, CD45RA<sup>+</sup>/Kg was a median of  $6.15 \times 10^3$ /Kg (range  $0-498 \times 10^3$ /Kg) and a median 4.70 (range 2.21–6.37) depletion log of CD45RA<sup>+</sup> cells. Median dose of CD45RO<sup>+</sup> cells (memory T-cells) infused was 8 (range 3.8–102)  $\times 10^7$ /Kg. Seventeen patients achieved neutrophil engraftment at median of 10 days (range 8–12) post-transplant. One patient could not achieve engraftment, died at day +8 due to sepsis. Two patients presented secondary graft failure (day +18 and +20), both received a second HSCT. Three patients developed aGvHD > grade II with gastrointestinal tract involvement, all steroids responsive. Three patients presented clinical features of cGvHD. Patients have an extensive skin involvement, with hepatic findings in one and pulmonary affection in other, at day +315, +130 and +330 post. Ten of 18 patients (55.5%) remain alive in remission with median follow-up 156 (range 8–597) days post-transplant. Eight patients died, 3 due progression at day +128, +117, +162 (2 presented positive minimal residual disease at HSCT), 4 due to infectious complications (days +8, +44, +50, +55) and 1 due to cardiogenic shock at day +253. Four patients relapsed, 3 of them died afterward with progressive disease. T-cells led immune recovery, achieved values higher than 500, 600, 1500 and 2400 cells/mL at day 30, 60, 90 and 210 respectively. Most of T cells were CD8+CD45RA<sup>-</sup> (median of 288, 370 and  $2334 \times 10^6$ /mm<sup>3</sup> respectively on day +30, +60 and +90) and CD4+CD45RA<sup>-</sup> T cells (median of 129, 161 and  $767 \times 10^6$ /mm<sup>3</sup> respectively on day +30, +60 and +90), while CD8+45RA<sup>+</sup> and CD4+45RA<sup>+</sup> cells remained low recapitulating the CD45RA depleted graft composition. Six patients presented cytomegalovirus reactivation, one progressed to CMV disease. Five patients with HHV-6 encephalitis. Probable aspergillosis in 1 patient (AML-M7 with secondary graft failure) at day +16 after second HCST. Two cases of toxoplasmosis (1 CNS, 1 pulmonary). CD45RA-depleted haplo-HCT showed acceptable tolerability with rapid and sustained engraftment as well as a full donor chimerism, minimal risk of acute GvHD and accelerated immunologic reconstitution. To note the high incidence of HHV-6 encephalitis seen.

**Disclosure of conflict of interest:** None.

#### P451

##### Collection of peripheral blood stem cells in teenager sibling donors: a single center experience

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Human leukocyte antigen (HLA) compatibility is important in allogeneic haematopoietic stem cell transplantation in order to reduce post-transplant complications; however, siblings only present a 25% chance of HLA-match with the patient. The well-known advantages and the low risk of complications associated to peripheral blood stem cells (PBSC) collected by apheresis made this procedure the first option in teenagers. The aim of this retrospective study was to analyse and characterize the paediatric sibling PBSC donor population assuring safety during the collection procedure, providing a high-quality product and accomplishing patient needs. We consulted the clinical files of donors under 18 years old since 1995–2015; a database in Excel<sup>®</sup> was created to register population characteristics, collection parameters and graft requirements. The informed consent was obtained from parents before procedure. The leukapheresis were performed with a COBE Spectra System; since 2009, we use a Spectra Optia Apheresis System. The donor/patient weight ratio (proposed by Styczynski *et al.*) was determined for each pair. The collection was programed based on clinical and analytical donor's features as well as transplant requirements. The analytical assays were done by a certified laboratory. We performed 29 PBSC apheresis in 23 healthy donors, 10 females and 13 males (Table 1). All of them started on the 5<sup>th</sup> day after mobilization with granulocyte colony-stimulating factor

(G-CSF) administered subcutaneously, bidaily. The weight ratio was < 1 in eight situations. Most of donations were performed by peripheral vein; a central venous catheter (CVC) was placed into a femoral vein in six adolescents. A median of 4 (3–5) blood volumes were processed during 174(115–318) minutes; the anticoagulation used was citrate+heparin (ratio 25:1). In general, one-collection day was enough to obtain the number of CD34<sup>+</sup> cells required; six donors had to perform a 2<sup>nd</sup> collection. In 19 cases, we cryopreserved the exceeding cells after graft infusion. The procedure was well tolerated, with only 2 adverse reactions registered (one hematoma in the puncture local; one paraesthesia due to hypocalcaemia induced by citrate). No blood products were used after the procedure or needed for the priming of the extracorporeal circuit. So far, no serious long-term adverse events were observed. Table 1. Median (range) of donors and leukapheresis products data. Our long lasting experience shows that PBSC collection in the teenage population is safe and feasible, allowing us to obtain a high-quality product for the patients. There were no adverse events associated with the G-CSF mobilization or CVC placement which is different from the experience of other groups. We recognize that leukapheresis by peripheral vein is a lengthy procedure but no complaint was reported to the collection team.

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[P451]

Donor characteristics	
Age (years)	16 (13-17)
Weight (kg)	58 (41-85)
G-CSF dose (µg/kg) §	13.33 (10-19,6)
CD34+ cell number (x10 <sup>6</sup> /L) ¶	71,5 (30-172)
Cellular therapy products	
CD34+ cell number (x10 <sup>6</sup> /kg)	10,10 (4,37-33,23)
CD3+ cell number (x10 <sup>6</sup> /kg) †	4,74 (1,94-17,45)
CFU-GM number (x10 <sup>5</sup> ) ‡	>500 (174->500)
Number of clinical files with unavailable data: §6, ¶7, †9, ‡12	

**Disclosure of conflict of interest:** None.

#### P452

##### Correspondence between clinical and histological grading of gastro-intestinal acute graft versus host disease in children

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Diagnosis of gastro-intestinal acute graft versus host disease (GI-aGvHD) is frequently confirmed by apoptosis findings on mucosal biopsies.<sup>1</sup> Aims of this single center retrospective study is to evaluate the correlation between clinical and histological grading of GI-aGvHD in children undergoing allogeneic haematopoietic stem cell transplantation (allo-HSCT), and to describe histological findings obtained by GI

endoscopies in order to evaluate usefulness in the diagnosis of GI-aGVHD. 348 allo-HSCTs were performed in our department between January 2000 and December 2015. GI biopsies were performed in 26 pts (7.4%) because of suspected GI-aGVHD. 14 pts were transplanted for malignant (53.8%) and 12 for not malignant diseases. The median age at HSCT was 9.5 years (0.5–16.9). 14 pts (54%) received myeloablative and 12 (46%) reduced intensity conditioning regimen. 21 pts (80.7%) received an unrelated donor (UD), 4 pts a related donor (RD) (15.3%), and 1 an haploidentical donor (3.8%). At onset of diarrhea, microbiological examinations of stool were performed and PCR research for CMV, Adenovirus, HHV6, EBV were evaluated in blood and in mucosal biopsies. Mucosal biopsies were obtained with esophago-gastro-duodenoscopy in 4 pts (15.3%), esophago-gastro-duodeno-colonoscopy in 3 (11.5%), pancolonscopy in 11 (42.3%), flexible sigmoidoscopy in 3 (11.5%), and rectal suction biopsy in 5 pts (19.2%). All mucosal biopsies, except in case of rectal suction, were obtained under sedation. The interval between mucosal biopsies and onset of GI acute symptoms was 23 days (from –66 to 103 days). Biopsies were taken from different sites in the GI tract, were stained using hematoxylin–eosin and evaluated using histological grading of aGVHD.<sup>1</sup> In these 26 pts the maximum grade of aGVHD was: grade 2 in one (4%), grade 3 in 14 (54%), and grade 4 in 11 pts (42%). At time of histological evaluation, diarrhea was the most common GI symptom (84.6%); 2 children had also cutaneous aGVHD and 5 hepatic aGVHD. PCR-CMV was positive in 2 mucosal biopsies obtained with pancolonscopy, PCR- Adenovirus in other 2 obtained with upper and pancolonscopy, PCR- HHV6 in 2 rectal biopsies, and PCR- EBV in one with upper and pancolonscopy. The comparison between clinical and histology grading of GI-aGVHD is shown in Table 1. Mucosal biopsies were positive in 1/4 pts evaluated with esophago-gastro-duodenoscopy (25%) (grade 1 aGVHD), in 3/3 pts undergone esophago-gastro-duodeno-colonoscopy (grade 1 in 2 and grade 3 in 1), in 8/11 (73%) who received a pancolonscopy (grade 1 in 5, grade 2 in 1, grade 4 in 2), and 7/8 (87%) of rectal biopsy obtained by sigmoidoscopy or rectal suction biopsy (grade 1

in 3, grade 2 in 1, grade 3 in 1, and grade 4 in 2). One patient developed duodenal intraparietal hematoma after upper endoscopy. In our experience, we did not demonstrate an overall correlation between clinical and histological grading of aGVHD showing that histological examinations underestimated the grade mild or moderate of aGVHD. We confirmed<sup>2,3</sup> that rectal biopsies represent to be more effective and safe diagnostic method for the confirm of diagnosis of GI-aGI.

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[P452]

**Table 1** : Correlation between histological and clinical findings of GI-aGVHD

Histological grade	Clinical GI- aGVHD			
	1	2	3	4
1 (n=11;42.3%)		5	2	4
2 (n=2;7.6%)				2
3 (n=2;7.6%)				2
4 (n=5;19.2%)			2	3
<b>Aspecific (n=7;26.9%)</b>	1	5		1
	1(3.8%)	10(38.4%)	4(15.3%)	12(46.1%)

**Disclosure of conflict of interest:** None.

**P453**

**Current role of hematopoietic cell transplantation in primary immunodeficiencies: a systematic review**

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During the past few decades, hematopoietic cell transplantation (HCT) as a treatment modality for Primary Immunodeficiencies (PID) has undergone remarkable advancement mainly due to better availability of alternate donors resulting in increase in not just matched unrelated donor (MUD) but also increased haploidentical (haplo) and cord blood transplants

[P453]

Number of studies	21				
Total Number of patients	1010				
Number of Haploidentical Transplants	317				
Conditioning	Myeloablative 476	RIC 121	No Conditioning 173	Other 39	NE (Not Evaluable) 201
Source of stem cells	BM 622	Cord 120	PB 107	Multiple/T-Cell depleted 12	NE 149
Acute GVHD	206/757				NE 254
Chronic GVHD	87/592				NE 418
Overall Survival	654/915				NE 95

(CBT). Additionally, refinement of the conditioning regimens and better graft versus host prophylaxis have presumably led to better survival outcomes. However, a literature gap is identified in evaluation of these outcomes in general with respect to donor and conditioning regimens. We conducted a systematic review by performing a comprehensive search of the PubMed and OVID library from its inception to August 2016. MeSH terms included 'Primary Immunodeficiency (immunodeficiencies)', 'stem cell transplant', 'bone marrow transplant' and 'hematopoietic cell transplant'. All PID studies which used HCT as a treatment modality were included. Experimental cellular therapies were excluded. Both cellular immunodeficiencies (e.g. SCID, WAS, A-T), and innate immunity disorders (e.g. IFNGR, CGD) were included in the search. Reviews, case reports, meta-analysis and non-English language articles were excluded from our electronic search. Publication bias was excluded by performing a methodological search of unpublished conference abstracts from the annual meetings of CIS, ASPHO, ASBMT, EBMT, and SIOP from 2000 to 2016. The data were analyzed considering the outcomes - overall survival and GVHD. 21 studies fulfilled the strict selection criteria for the electronic search comprising of 1010 PID patients. In majority of the HCTs, a myeloablative conditioning regimen (MAC) was utilized (47% of the evaluable) but a shift towards more reduced intensity conditioning (RIC) was observed in the later years. 120 CBTs were identified. 27% of patients developed some degree of acute GVHD, whereas chronic GVHD was identified in 15% of the patients. Total number of haplos was 317. Overall survival was found to be 71% post-HCT. A meta-analysis could not be performed due to the heterogeneity of both the predictor variable data (combined stem cell sources were also used for HCT) and due to the extremely small number of the patients when categorized in subgroups (e.g. for OMENN syndrome, RAG deficiencies). This is the largest study of HCTs in PID, and we observe that alternate donor HCTs have increased significantly over the past decade for the treatment of PID. While the incidence of chronic GVHD was low, acute GVHD still remains a problem in about a third of the PID patients transplanted.

**Disclosure of conflict of interest:** None.

#### P454

##### **Defibrotide for the prevention and treatment of hepatic veno-occlusive disease after hematopoietic stem cell transplantation: a single-center experience**

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Hepatic veno-occlusive disease (VOD) is a common and serious complication of hematopoietic stem cell transplantation (HSCT) in children. We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT. In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25 mg/kg per day in four divided intravenous infusions over 2 h, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 20 days as a prophylaxis. In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1–20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenitale dyserythropoetic anemia, one patient with osteopetrosis, four patients with famial hemophagocytic lymphohistiocytosis, two patients with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were seen. In eight patients developed clinical VOD (Seattle criteria). In these patients, defibrotide dose was increased to a treatment dose of 40–60 mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who

developed VOD are being well and no patient have transplant related complications. Hepatic veno-occlusive disease, which is caused by hepatocyte and sinusoidal vessel endothelium damage, can occur early after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multiorgan failure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

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**Disclosure of conflict of interest:** None.

#### P455

##### **Dynamic models of immune reconstitution to predict the risk of infections after allogeneic hematopoietic stem cell transplantation**

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Immune reconstitution (IR) is critical for clinical outcome after allogeneic hematopoietic stem cell transplantation (HSCT). Host and proceeding-related factors affect the IR dynamics and survival. Isolated IR parameters are commonly correlated and proposed to predict clinical outcomes after HSCT, but these approaches only confer prognostic value at single time points or for single markers. We aim to demonstrate an appropriate methodology to assess the capability of combined serial measurements of lymphocyte subsets to reflect the impact of infections on IR after paediatric HSCT. Retrospective data of patients receiving a first HSCT for any indication with any cell source in the paediatric HSCT program from 2006 to 2015 were included. To characterize the kinetics of immune reconstitution, CD3+, CD4+, CD8 + T-cells, B-cells, NK-cells and their naive and memory subsets were measured and analysed at various time points at 2 years post-HSCT to establish a joint model for the evolution of cell subpopulations. Slope per month (cellular increase or decrease) of each lymphocyte subsets were calculated and compared with clinical outcomes and cumulative risk of infections. A total of 88 children (range from 0–15 y.o. median 5 y.o.) were included, with CB (*n* = 19) PB (*n* = 22) and BM (*n* = 47) as cell sources. The cumulative incidences after early period were 45% for viral infections (EBV 27%, CMV 22%, BK 11%, Adv 4%) and 30% for bacterial infections. Data on IR were available for 77% of the disease-free survivors. In a exploratory multivariate analysis we detected mainly differences in CD8+, CD8+CD45Ro+ memory and NK cells at 1 year after HSCT, with dependent tendency according on the cell source and HLA compatibility. Analysis of the slope tendency patterns were established for the analysis of

the impact of infections in the IR. Delay in CD8+ and CD8+Ra+ appearance (mean slope/m = -7.1% and -1.8% respectively) remarks the IR profile for bacterial infections, and delayed in NK, CD8 and CD8Ro+ (-8.4%, -5.8%, -26% respectively) for overall viral infections. Additional correlations allow differences in EBV (CD8+Ra+ high mean slope/m = 15.9%), CMV (delayed in CD8Ro+ slope/m = 10%), and BK infection (CD8+Ra + plus CD4Ro+ and NK high mean slope/m = 24.9%, 36% 22%). Understanding the dynamics of reconstitution by integrating information from the monitoring of lymphocyte subpopulations allows the establishment of kinetic profiles that may help to evaluate the risk of infections and adjust infection prophylaxis in the follow-up of transplanted patients.

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**Disclosure of conflict of interest:** None.

#### P456

##### Early CPAP in the ward prevents evolution of acute lung injury in pediatric HSCT patients

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Mortality rate in HSCT patients admitted to intensive care unit (ICU) is still as high as 20% to 70%. This rate increases when respiratory complications progress to acute respiratory failure (ARF) requiring mechanical ventilation (MV).<sup>1</sup> The aim of this study was to determine the feasibility and effectiveness of early continuous positive airway pressure (CPAP) delivered in a pediatric hematology-oncology ward to prevent occurrence of ARF requiring MV. We retrospectively analysed children treated with CPAP in our pediatric hematology-oncology ward between October 2011 and October 2016. Thirty-two patients received CPAP delivered with helmet during the study period. Data were available for 26 patients, 15 males and 11 females, median age 12 years [range 2–20]. Eighteen patients underwent allogeneic HSCT: 1 from sibling donor, 11 from matched unrelated donor, 4 from haploidentical family donor, 2 from cord blood unit. Seven patients had a malignant disease: 5 ALL, 1 AML, 1 Ewing sarcoma. Infectious pneumonia was the main cause of ARF in 16/26 patients (61.5%): 9 viral pneumonitis (4 Rhinovirus, 3 Parainfluenzae virus, 1 Respiratory Syncytial Virus and 1 CMV). Five patients had proven/probable invasive fungal infection according to EORTC criteria (3 aspergillosis and 2 mucormycoses). Other causative agents were *Pneumocystis jirovecii* (1), *Bacillus* of Calmette and Guerein (1), *Toxoplasma gondii* (1) and *St. mitis* (1). Non infective causes of ARF were acute transfusion related lung injury (2), hemorrhagic alveolitis (2), cryptogenic organizing pneumonia (3), tumor lysis syndrome (1), and alveolar oedema due to renal failure (1). According to chest imaging, 13/26 patients (50%) presented with pulmonary consolidations, while 31% had both interstitial infiltrates and pulmonary consolidations. At baseline median neutrophil count was  $2.05 \times 10^3/\mu\text{L}$  (range  $0-21.0 \times 10^3/\mu\text{L}$ ), mean heart rate 128 bpm, pulsioximetry saturation in room air 86%. H-CPAP was applied in 19/26 patient with a curative aim, in 7/26 patients as palliative support to reduce respiratory distress. Median positive pressure delivered was 10 cmH<sub>2</sub>O (7–12 cmH<sub>2</sub>O), median FiO<sub>2</sub> was 40% (30–100%). H-CPAP was applied for a median of 11 days (4–34). No patient failed H-CPAP because of agitation or adverse events (skin breakdown, conjunctivitis, gastric distension or epistaxis). Ten patients were transferred to ICU (34.6%), 8/10 because of HSCT complications. Median ICU stay was 8.7 days (2–20). Only 3 patients required mechanical ventilation, in 2 cases associated to ECMO. Nether pSaO<sub>2</sub> in room air ( $P$  0.98 CI 95%) nor CPAP level ( $P$  0.76 CI 95%) correlated with the need of ICU admission. Patients

requiring higher FiO<sub>2</sub> during CPAP demonstrated a not statistically significant trend to higher ICU admission rate ( $P=0.149$ ). There was a higher rate of MV in patients with higher CPAP FiO<sub>2</sub> level ( $P=0.008$ ). MV prolonged ICU stay ( $P$  0.0049). Cumulative mortality was 34.6% (9/26); only 1 patient died in ICU (10%), because of post HSCT Parainfluenza virus pneumonitis requiring ECMO. Helmet CPAP delivery in pediatric HSCT ward is feasible and safe, both for curative and palliative aim. If applied early, CPAP could reduce ICU admission rate for MV and ICU mortality.

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**Disclosure of conflict of interest:** None.

#### P457

##### Effectivity of defibrotide prophylaxis on acute graft versus host disease: a single center experience with high risk pediatric patients

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Veno occlusive disease (VOD) and graft versus host disease (GvHD) are both dreadful complications of hematopoietic stem cell transplantation (HSCT). Although they have different clinical signs, it is suggested that they share similar pathophysiological pathway. Defibrotide is used in the treatment of VOD for a long time but it is very less known about its effect on GvHD. In this study, we analyzed a 'high risk' patient population in pediatric HSCT to show the effect of defibrotide on acute GvHD. Between June 2014–August 2016 totally 75 'high risk' pediatric allogeneic HSCT procedures were enrolled in this study. 'High risk' definition involves busulphan/melphalan usage in conditioning regimen, second myeloablative HSCT, pre-existing liver disease, allogeneic HSCT for leukemia with second relapse and diagnosis of hemophagocytic lymphohistiocytosis (HLH) or osteopetrosis. Defibrotide prophylaxis group ( $n=22$ ) received 25 mg/kg/day per day and continued for at least 14 days after transplantation. The control group ( $n=53$ ) received only continuous infusion of low-dose heparin until 30 days after transplantation. For the comparison between groups, the Fisher's exact test was used. All analyses were performed using SPSS 20 and  $P$ -value of 0.05 was considered statistically significant. We analyzed totally 75 HSCT procedures with different diagnosis; 17 beta thalassemia major, 14 leukemia, 9 HLH, 18 primary immunodeficiencies, 3 osteopetrosis, 4 Fanconi aplastic anemia (FAA), 2 myelodysplastic syndrome, 2 neuroblastoma, 1 congenital amegakaryocytic thrombocytopenia, 1 Krabbe disease, 1 aplastic anemia, 1 hypereosinophilic syndrome and 1 sickle cell disease. All the procedures meet the 'high risk' definition; most of them ( $n=50$ ) have busulphan for conditioning, also there are 9 HLH and 3 osteopetrosis patients, 2 neuroblastoma patients had the second myeloablative regimen, FAA and aplastic anemia patients had pre-existing liver disease, and 2 of the leukemias had beyond second relapse. The mean age was 6.7 years old (0.25–19.6), 27 HSCT performed from match sibling donor (MSD) (36%), 3 HSCT from match family donor (MFD) (4%), 43 HSCT from match unrelated donor (MUD) (57%) and 2 HSCT from haploidentical mother (3%). We especially focused on GVHD and VOD. Totally 13 VOD cases (17%) in these 75 HSCT procedures were detected. Only two of them detected in the prophylaxis group (9%) and 11 cases in the control group (20%). There were 32 CMV reactivation cases detected in 75 HSCT procedures (42%). In the prophylaxis group there were 11 cases (50%) and in the control group 21 cases (39%). We detected 36 acute grade I–IV GvHD cases in 75 HSCT procedures (48%). Only 4 of them were in the prophylaxis group (18%) and 32 cases were in control group (60%). The prophylaxis group's aGvHD ratio was significantly lower than the control group ( $P=0.001$ ). Defibrotide for VOD prophylaxis

is confirmed by several studies, but its benefits for aGVHD is not clear. In this study, we show the significant effect of defibrotide on aGVHD. We speculate that the protective effect of defibrotide on both VOD and aGVHD could be explained by the similar pathophysiology of these complications. We need larger studies on the pathophysiological pathways, then we could invent more effective interventions.

**Disclosure of conflict of interest:** None.

#### P458

##### **First use of mini buffy coat extracorporeal photopheresis for the treatment of graft-versus-host-disease in a low body weight pediatric patient in Croatia**

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Conventional extracorporeal photopheresis (ECP) has proven efficient for the treatment of graft-versus-host-disease (GVHD) but is limited to patients with sufficient body weight. A mini buffy coat ECP (mini-ECP) 'off line' technique that allows treatment of small children has been developed, using various methods for mononuclear cell (MNC) separation from whole blood. We present treatment of low body weight child with mini-ECP 'off line' technique using Sepax System for mononuclear cell (MNC) selection and Macogenic irradiator. A toddler with juvenile myelomonocytic leukemia (JMML) developed acute GVHD, after a matched unrelated stem cell transplantation (SCT) performed at the age of 12 months. Acute GVHD of the skin occurred three months after SCT and responded to high dose steroids, but recurred six months after SCT (biopsy of the skin confirmed acute GVHD) together with GVHD of the liver. Because of the resistance to steroids and cyclosporine, mini-ECP was introduced as therapy. The patient weighed 8 kilograms. Blood was collected from tunneled central venous catheter, and collected volume was replaced with saline infusion. The cord blood collection bag (Macopharma, France), which contains 21 mL citrate phosphate dextrose (CPD) anticoagulant solution was used for whole blood collection. Whole blood was processed using Sepax System separator (Biosafe, Switzerland), and final volume of buffy coat was set on 25 mL. Extracted buffy coat was transferred into the UV-A Illumination Eva Bag (Macopharma, France) and diluted with saline solution up to 200 mL. 8-methoxypsoralen (Gerot, Austria) was injected directly into the UV-A illumination bag, and cells were irradiated by the UV-A illumination device Macogenic 2 (Macopharma, France). Irradiated cells and autologous residual blood were reinfused back to the patient. During the whole procedure patient's vital signs were monitored. ECP procedures were performed 3 times per week for 4 weeks, followed by 2 times per week at 2 weeks intervals for 2 months. In 3 month period 28 mini-ECP procedures were performed. Median of collected whole blood was 92 mL (range 52–100). Median of total nucleated cell (TNC), and mononuclear cell recovery after Sepax separation were 88.3% (range 66.7–104), and 90.8% (range 63–102), respectively. Median of hematocrit in final irradiated product was 4% (range 3.8–6%). Patient was reinfused with median of 1.0 (range 0.66–1.8) TNC  $\times 10^8$ /kg BW, and median of 4.95 (range 3.62–13.72) lymphocyte  $\times 10^8$ /kg BW. After one month of ECP together with steroids and cyclosporine, GVHD of the skin improved, and the steroids were gradually weaned, with no recurrence. GVHD of the liver showed no improvement, and other therapies had to be introduced, but without steroids. For the first time in Croatia, mini-ECP was performed in a child with GVHD, in whom conventional ECP could not be used because of insufficient body weight. MNC separation using automated closed system Sepax separator has proven efficient and safe. Mini-ECP treatment was continued for three months, without technical difficulties. Positive effect was experienced concerning the skin GVHD, but not the liver GVHD. After the first experience in our country, in future we

plan to use this technique for low-weight patients or patients with contraindications for apheresis, which are in need of second- or third-line therapy for GVHD.

**Disclosure of conflict of interest:** None.

#### P459

##### **Gonadal function after Busulphan and Treosulfan in children and adolescents undergoing allogeneic haematopoietic stem cell transplantation. On behalf of paediatric and complications and quality of life EBMT working parties**

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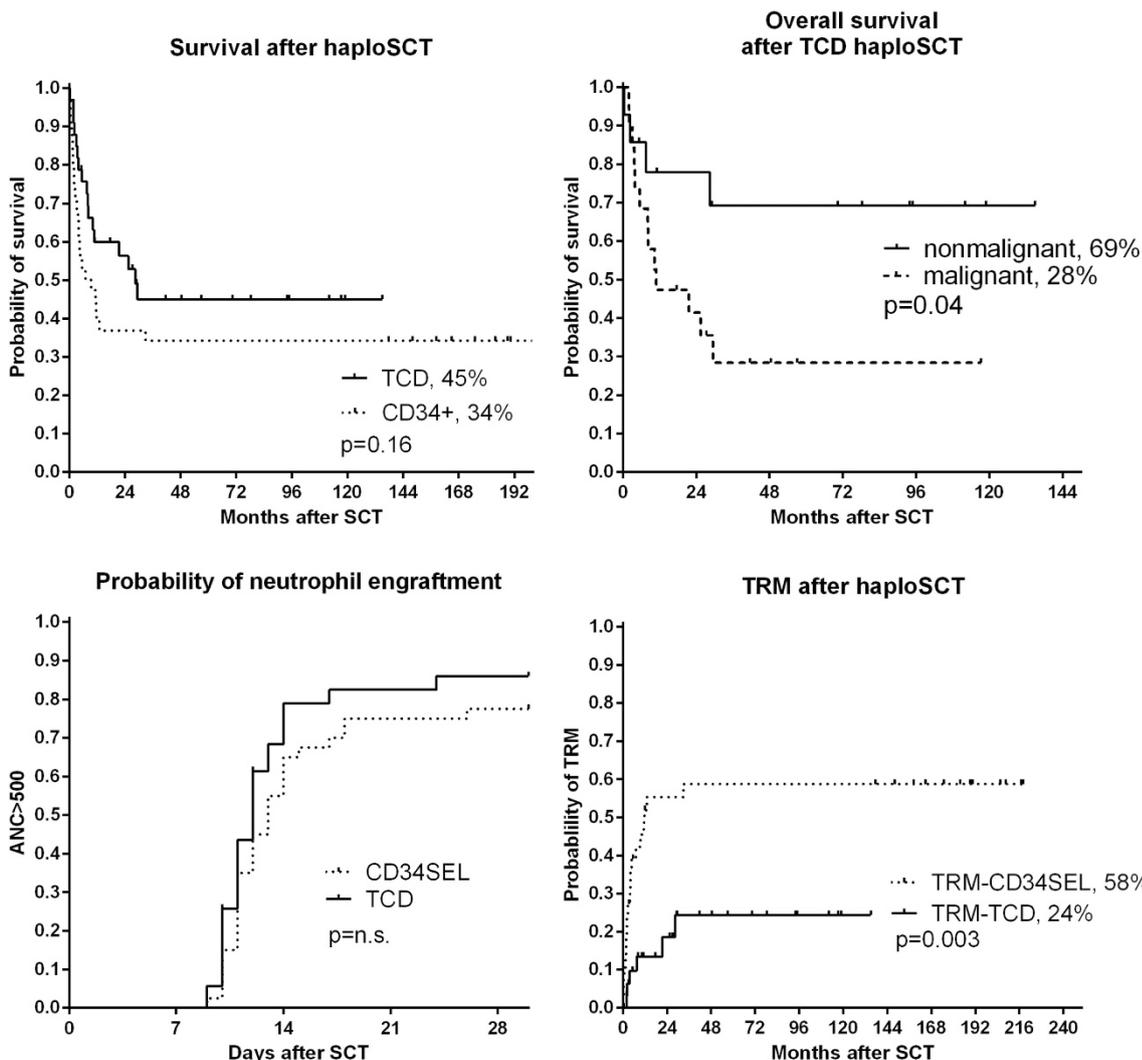
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Gonadal failure represents one of the late effects of haematopoietic cell transplantation (HSCT) with a negative impact on quality of life in young patients (pts) undergoing HSCT.2. The aim of this retrospective multicentre EBMT study was to assess gonadal function in untreated pts undergoing allogeneic HSCT between 5 to 20 years (yrs) of age, after a preparative regimen with Busulphan (BU) or Treosulfan (Treo). Eighty-seven pts (32 females, 55 males) were reported from 17 out of 123 contacted EBMT centers: 26/87 (30%) received allogeneic HSCT during pre-pubertal and 61/87 (70%) in pubertal phase. Of the 87 pts, 76 (87.4%) received BU in myeloablative dose [25 pre-pubertal, (median age of 6.7 yrs) and 51 pubertal, (median age of 13.4 yrs)] and 11 pts (12.6%) received Treo (1 in pre-pubertal and 10 in pubertal period). Underlying diseases were primary immunodeficiency (34.5%), chronic myeloid leukemia (33.3%), myelodysplastic syndrome (24.1%), familial haemophagocytic lymphohistiocytosis (6.9%) and Shwachman-Diamond Syndrome (1.1%). 17/26 of pre-pubertal pts (71%) developed spontaneous puberty (69.5% in the BU group and 100% in Treo group). 21/28 (75%) females undergoing HSCT during puberty completed their pubertal development (71.4% in BU group and 100% in Treo group). None of females (4/4) with BU during pre-pubertal phase developed spontaneous menarche (SM), while 33.3 % (7/21) of females who received BU in pubertal period had SM. All females (n=5) treated with Treo during pubertal phase had SM (100%). For both conditioning regimens, the 42.8% (12/28)



of females treated during the puberty experienced SM. Among the remaining 14 females (for 2 pts the information is missing) who did not developed SM, 13 received HRT 2.5 yrs after HSCT and 5 of them had ovarian recovery after a median of 2.3 yrs from HSCT (1.43–6.72). The median age at last follow up was 15.8 and 13.2 yrs in BU and TREO pre-pubertal group, and 22.2 and 19.9 yrs in BU and TREO pubertal group respectively. In the pubertal group, 18 females (69.5%) are still receiving hormonal replacement therapy (HRT) (16 in the BU group and 2 in TREO group). 2 pts (7.4%) had spontaneous pregnancy. No problems in newborns are reported. Sperm analysis was performed in 18.2% of pubertal pts (6/33) of males, and 66% (n=4/6 treated with BU) were azoospermic (data regarding 2 pts were missing). The sperm analysis was repeated in half of the males. Until now no paternity was reported. In this experience, the pubertal development in pts who received TREO (n=6) was normal, and in the BU group the majority of females (70%) had normal puberty. The rate of SM is higher (100%) in females after TREO than BU (28%). The HRT is ongoing at last follow-up in 76% of females treated with BU and in 40% of those who received TREO. Our data suggests that TREO may have a better outcome than BU in young girls receiving allogeneic HSCT and larger studies are warranted. Male patients require longer follow-up.

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**P460 Haploidentical SCT in pediatric patients: a single-centre experience**

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Immunomagnetic CD34 enrichment (CD34SEL) and T-cell depletion (TCD) remain the most efficient methods of GVHD

prevention in patients transplanted from partially matched donors. We report the single centre experience in haploidentical SCT. In years 1999–2016 in the Department of Pediatric BMT, Oncology and Hematology at Wrocław Medical University, 72 children underwent SCT from partially matched, haploidentical parental donors. Graft manipulation in 38 patients consisted of CD34SEL, in 22 patients the CD3 immunomagnetic depletion (TCD-CD3) was performed, and in 12 – TCR alpha-beta depletion (TCD-AB). We analysed the impact of type of manipulation procedure, conditioning regimen, demographic factors, and KIR genotype on survival and probability of neutrophil recovery. The probability of engraftment and neutrophil recovery was 86% vs 77% in CD34SEL group ( $P=NS$ ). Probability of 5 year overall survival in the TCD group was similar to the CD34SEL group (45% vs 34%,  $P=NS$ ). In the TCD patients, neither use of busulfan vs treosulfan, nor KIR genotype, nor donor sex had noticeable impact on SCT result and survival. Patients transplanted after TCD due to non-malignant disease had higher survival probability, than those with malignancies (69% vs 28%,  $P=0.04$ ). The TRM in TCD patients was reduced in comparison to CD34SEL (24 vs 58%,  $P=0.003$ ). The TRM after TCD resulted mostly from severe viral infections in TCD-CD3 patients. In 4/34 TCD patients spontaneous acute, skin (stage 2) GVHD was diagnosed and successfully treated. Two patients received unmanipulated donor lymphocyte infusions (DLI) and developed severe acute steroid-resistant (grade 4) GVHD, in one of them with fatal outcome. TCD methods are superior to CD34SEL due to significant reduction in treatment related mortality. Haploidentical SCT after TCD can result in durable engraftment, but warrants intensive post-transplant monitoring for infectious complications and cautious approach to DLI therapy.

**Disclosure of conflict of interest:** None.

#### P461

### Haploidentical stem cell transplantation with post-transplant cyclophosphamide or *in vivo* $\alpha\beta$ +T and CD19+B cells depletion: a single-center pediatric experience

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HLA-haploidentical stem cell transplantation (HAPLO) is a valuable option for children requiring allogeneic transplant. The aim of this study is to report the Gaslini Institute experience with two platforms of HAPLO in the last 4 years: *in vivo* post-transplant cyclophosphamide (PTCy) (Luznik-BBMT 2008; Sanz-BMT 2012), *ex vivo* depletion of donor  $\alpha\beta$ +CD3+ and CD19+Bcells (Bertaina-Blood 2014; Schumm-Cytotherapy 2013). **Patients and methods:** from 11/2012 to 09/2016, 26 children with either malignant (15; 57.7%) or non-malignant diseases (11;42.3%) received HAPLO at Gaslini. Fourteen patients (53.8%) received PTCy and 12 (46.2%) $\alpha\beta$ +CD3+/CD19+depleted graft (CliniMACS device). Among malignancies, 9 (60%) were transplanted in complete remission (6ALL, 3AML) and 6 (40%) with residual disease (1AML, 2MDS, 1LH, 2NHL). According to disease protocols, conditioning regimen was myeloablative (MAC) in 17 (65.4%), non-myeloablative (NMA) in 9 patients (34.6%). Table 1 summarizes patients, transplant features, GvHD prophylaxis. In  $\alpha\beta$ +/CD19+depleted group, PT-immunosuppression was not performed, except in 2 for  $\alpha\beta$ +CD3+cells exceeding  $1 \times 10^5$ /kg in the graft. **Results:** engraftment was achieved in 24 patients (92.3%) after a

[P461]

	Total (%)	PT-Cy	$\alpha\beta$ +CD19+ depleted (CliniMACS device)	P
M/F	10/16	5/9	5/7	1.00*
Median age at HAPLO (years)	9.9 (4.5-13.8)	12.9 (9.4-15.2)	6.4 (1.8-11.5)	0.02*
Malignancies:	15 (57.7)	13	2	
6 ALL 4 AML 2 MDS [1 RAEB-T and 1 transformed FA], 2 NHL 1 LH				
No malignancies:	11 (42.3)	1	10	<0.05*
5 PID [1 WAS, 1 TACI, 1 PI3K, 1 RAG1, 1 n.d.] 3 BMF (3 FA-related [not transformed], 1 DC, 1 CAMT) 1 SAA				
Stem cell source		14 (100) Bone marrow	12 (100) Peripheral blood	<0.05*
Cell dose (median)				
MNCs ( $\times 10^6$ /kg)			12.5 (4.7-19)	
CD34+ ( $\times 10^6$ /kg)	7.1 (1.9-19)	4.7 (1.9-9.7)	15.5 (6.3-24.7)	
CD3+ ( $\times 10^6$ /kg)	6.7 (1-24.7)	3.2 (1-6.7)	0.76 (0.41-0.92)	-
$\alpha\beta$ + ( $\times 10^6$ /kg)			0.53 (0.06-4.65)	
$\gamma\delta$ + ( $\times 10^6$ /kg)			134.8 (1.65-957.1)	
CD19+ ( $\times 10^6$ /kg)			0.19 (0.04-8.6)	
Conditioning regimen		10 → Valencia protocol, Sanz-BMT2013 4 → Baltimore protocol, Luznik - BBMT 2008 [G-CSF from d +5 until 3th ANC > 1000/mm <sup>3</sup> ]	7 5	0.68*
- Myeloablative - Non myeloablative	17 (65.4) 9 (34.6)			
Pre-transplant immunosuppression		None	ATG-Fresenius 4 mg/kg d 4/-3/-2 Rituximab 200 mg/mq d -1	-
Post-transplant pharmacologic GvHD prophylaxis		10 → Valencia protocol – Sanz-BMT2013: - PTCy 50 mg/kg d +3/+5; - CyA 1-3 mg/kg/d from d 0; - MMF 30 mg/kg/d from +1 to +35 4 → Baltimore protocol, Luznik-BBMT 2008: - PTCy 50 mg/kg d +3/+4; - CyA 1-3 mg/kg/d from d +5; - MMF 30 mg/kg/d from +5 to +35	9 → none 2 → CyA 1 mg/kg**	-
Total	26 (100)	14 (53.8)	12 (46.2)	

**Legenda:** ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; MDS – myelodysplastic syndrome; RAEB-T: refractory anemia with excess of blasts; NHL: non-Hodgkin lymphoma; LH: Hodgkin lymphoma; PID: primary immunodeficiency; BMF: bone marrow failure; SAA: severe aplastic anemia; FA: Fanconi anemia; WAS: wiskott-aldrich syndrome; MNC: mononuclear cells; PTCy: post-transplant cyclophosphamide; CyA: cyclosporine-A; MMF: mycophenolate; ANC: Absolute neutrophil count.  
\* Fisher's Test      \* chi quadro test      \* Non parametric Mann-Whitney Test  
\*\*  $\alpha\beta$ + T cells in the graft superior to  $1 \times 10^6$  after depletion

median of 15 days for neutrophils in both groups, 17 for platelets (23 in PTCy, 12 in  $\alpha\beta$ +CD3+/CD19+depleted,  $P$  0.005). Donor chimerism was complete in 22 patients (84.6%). In  $\alpha\beta$ +CD3+/CD19+depleted group, 4 patient rejected (33.3%:1 primary and 3 secondary reject, 22, 28, 55 and 195 days after HAPLO, respectively) and were rescued with a second transplant. Seven patients (50%) developed acute (a-) GvHD in PTCy group (grade 1–2 in 4; grade 3–4 in 3), compared to one (8.3%: grade 4) in  $\alpha\beta$ +CD19+depleted group ( $P$  0.02). Among patients at risk, 3 out of 9 in PTCy group developed chronic (c-) GVHD (33.3%:1 score-3, 1 overlap, 1 score-1), compared to 0/9 patients in  $\alpha\beta$ +CD3+/CD19+depleted group ( $P$  0.08). The cumulative risk of CMV-reactivation was 72% and 58% in PTCy and  $\alpha\beta$ +CD3+/CD19+depleted groups, respectively ( $P$  0.63). T-cell reconstitution was significantly different in the two groups, with a median absolute number of CD16+56+CD3- and  $\gamma\delta$ +CD3+ higher in  $\alpha\beta$ +CD3+/CD19+depleted group on day +120 ( $P$  0.03) and a median number of CD3+CD8+ higher in PTCy group on day+180 ( $P$  0.04). Length of hospitalization was shorter in the  $\alpha\beta$ +CD3+/CD19+depleted group, with a median time from HAPLO to discharge of 23 days compared to 31 days in the PTCy group ( $P$  0.003). After a median follow-up of 9 months (1–48), 20 patients (76.9%) are alive and disease-free. Five patients died in PTCy (35.7%:2 relapse, 2 sepsis, 1 HHV6-related limbic encephalitis), one (8.3%) died in  $\alpha\beta$ +CD19+depleted group (GvHD). The 3-year overall survival (OS) probability was 85% [CI95%:0.33–0.97] in  $\alpha\beta$ +CD3+/CD19+depleted group and 47% [CI95%:0.13–0.75] in PTCy group ( $P$  0.14). According to disease, OS was 75% [CI 95%:0.30–0.93] in non-malignant disease and 53% [CI 95%:0.15–0.81] in malignancies ( $P=0.68$ ). The 3-year transplant-related mortality (TRM) was 15% [CI 95%:0.03–0.67] in  $\alpha\beta$ +CD19+depleted and 37% (0.12–0.83) in PTCy group ( $P$  0.42). This experience confirms both platforms as feasible and effective options for HAPLO in children, with low incidence of severe a- and c-GvHD and low TRM. The lower incidence of a- and c-GvHD in  $\alpha\beta$ +CD3+/CD19+depleted group suggests this platform as a preferable option in patients with non-malignant disease.

**Disclosure of conflict of interest:** None.

#### P462

##### **Haploidentical stem cell transplantation in children; a single center experience of alpha beta T-cell depleted and unmanipulated transplantation with post-transplant cyclophosphamide**

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Some children have not donor and an urgent need to proceed to transplantation because of disease status. We reviewed the role of haploidentical transplantation in children and report our single center experience. Ten children were transplanted from haploidentical family members donors (median age:12.5 years). We performed alpha beta T cell depleted transplantation in three patients and unmanipulated bone marrow transplantation with posttransplant cyclophosphamide in seven patients. The diagnosis were eight high risk leukemias (three ALL and five AML) and two severe aplastic anemia. Patients were myeloablative conditioned with cyclophosphamide, fludarabine and total body irradiation in aplastic anemia received alpha beta T cell depleted grafts with a median CD34 cell dose of  $2.9 \times 10^6$ /Kg (range:2.6–3.2) and busulphan, cyclophosphamide in high risk leukemias received unmanipulated bone marrow grafts with posttransplant cyclophosphamide in 3rd and 5th day of posttransplant with a median CD34 cell dose of  $7.4 \times 10^6$ /Kg (range:2.38–16.1). Median follow up of our patients 10 months. Six of 10 patients are alive and in disease free follow up. Four patients were relapsed and dead median 5.5 months of transplantation. The rate of relapse was 50 % for leukemia patients in remission and 50% for patients with active disease. Myeloablative conditioning regimen followed by haploidentical bone marrow

transplantation with posttransplant cyclophosphamide may be an option in high risk leukemia patients need urgent transplantation because of disease status who have not donor.

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#### P463

##### **Haploidentical stem cell transplantation with post-transplant HD-cyclophosphamide in children—single center experience**

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Pts with high-risk hematological malignancies have a poor prognosis without a hematopoietic stem cell transplant. Haploidentical SCT is a potential cure for pts without HLA-identical donor and post-transplant HD-Cyclophosphamide (HD-CY) permits the use of T-cell replete grafts in settings where ex-vivo manipulation is not available. The experience with haplo-SCT with post-transplant HD-CY in the pediatric population is limited. We report our experience from 5 pts (2–15 y) with malignancies who underwent haploidentical HSCT with post-transplant HD-CY from April 2015 to June 2016. The different conditioning regimens are described in Table 1. All patients received HD-CY 50 mg/kg on D+3 and D+4. Cyclosporine A 3 mg/kg/d i.v., then 6 mg/kg/d p.o. adjusting for blood levels 200–400 ng/ml and mycophenolate mofetil 15 mg/kg 2 times daily po were started on D+5. MMF was discontinued on D+35, CsA- after D+100. All pts received anti-microbial prophylaxis for bacteria, fungal, herpes infection and Pneumocystis according to institutional practices. Analysis for donor chimerism and MRD were performed at D+30, +60, +100, +180. Pts, donors and stem-cell harvest characteristics are described in Table 1. 4 pts had high risk hematological malignancies, and 1 relapsed after auto-SCT neuroblastoma (HR-NB). 1 pt was transplanted in 1st CR (AML M7) and others in 2nd CR. 3 pts had full engraftment (neutrophil engraftment at 17,18 and 22 days). 1 pt (HR-NB) was concerned as a primary failure for achieving neutrophil and platelet engraftment by D+30, despite of complete donor chimerism in bone marrow. He was transplanted additionally with the same donor at D+49 after 1st transplant. 1 pt died before engraftment at D+20 (fulminant Ps. Aeruginosa-sepsis). 2 pts remain alive in CR (2ndCR-AML and 1st CR-M7 AML) with follow-up of 375 and 164 days (05/12/2016) without cGVHD with complete donor chimerism. 2 pts relapsed after D+100 (were transplanted in 2nd CR-FLT3 AML and 2nd CR-NB) and died. 1 pt died because of infectious complication at D+20 (transplanted in 3d CR-ALL). 4/5 pts had grade 2 acute GVHD. HLA-haploidentical HSCT with post-transplant T-cell *in vivo* repletion grafts by using HD-CY is feasible and effective in children with HR-hematological malignancies.

[P463]

Table 1. Pts, donor and harvest characteristics

Variable	N=5
Age, median (range in years)	11 (2-15)
Gender,n	
Male	4
Female	1
Diagnosis,n	
ALL-3 CR	1
AML- 1 CR	1
AML-2 CR	2
NB-2 CR	1
Conditioning regimen,n	
Flu 150mg/m <sup>2</sup> + Treo 36g/m <sup>2</sup> +Mel140 mg/m <sup>2</sup>	1
Flu 150mg/m <sup>2</sup> + ARA-C 8000 mg/m <sup>2</sup> +Mel 180 mg/m <sup>2</sup>	1
FLAME- Flu 150 mg/m <sup>2</sup> +CCNU 300mg/m <sup>2</sup> + ARA- C 4000 mg/m <sup>2</sup> + VP-16 1000 mg/m <sup>2</sup> +Mel 140 mg/m <sup>2</sup>	3
Donor,n	
Father	3
Brother	2
Stem cell source	
PB	3
BM	2
Infused	
PBSC: CD34+ x 10 <sup>6</sup> /kg	5.8;15.16;21.48
BM : volume ml/kg	18;18
TNC x10 <sup>8</sup> /kg	1.28;3.37
CD34+x10 <sup>6</sup> /kg	0.61;1.6

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### P464

#### Previously published

### P465

#### Hematopoietic stem cell transplantation in children with thalassemia major: the experience of single center

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Beta-thalassemia major genetic defect causes reduced or absent beta globin synthesis resulting in an imbalanced accumulation of alpha-globin chains and ineffective erythropoiesis with hemolysis. Hematopoietic stem cell transplantation (HSCT) remains the only cure for thalassemia major at this time. We aimed to present the results of HSCT for children with beta-thalassemia major at Acibadem Adana Hospital Pediatric Bone Marrow Transplantation Unit. Between 2012 and 2016, 51 patients with thalassemia major received an allogeneic BMT at Acibadem Adana Hospital Pediatric Bone Marrow Transplantation Unit. Medical records, complications and treatment modalities were evaluated. Between 2012 and 2016, 51 children of which 31 male and 21 female underwent allogeneic BMT in our center. The median age at BMT was 7.4 years. Using the Pesaro risk classification, 29 patients were class 1, 15 were class 2 and 7 were class 3 respectively. The median serum ferritin level was 1695 ng/mL. Seventeen patients received bone marrow stem cells; 29 peripheral blood stem cells (PBSC), 3 cord blood stem cells, 2 cord blood stem cells and bone marrow stem cells. Engraftment times were shorter with patients who underwent PBSC. The donors were HLA-matched sibling or parents except for two donors

who were match unrelated donor. Thalassaemic reconstitution occurred in three patients. Acute graft-versus-host disease (GVHD) of grade II–IV occurred in 17 % and chronic GVHD in 12%. Acute and chronic GVHD were seen more frequently in patients with class 2–3 compared to class 1. Mortality rate was also higher in these groups. Seven patients died. One patient died on post-transplant day 26 due to intracranial bleeding. The other 6 patients with chronic GVHD died between 182 and 257 days, on average 219 days post-transplant. These data suggest that allogeneic BMT remains an important treatment option for children with beta-thalassaemia major, particularly when compliance with iron chelation is poor.

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#### P466

##### Hematopoietic stem cell transplantation in MAHAK, (NGO)/2016 report

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The Society to Support Children Suffering from Cancer, also known as MAHAK, was set up in 1991 as a non-governmental and non-profit organization. In the past two decades, the organization has attracted a vast public support and fulfilled a great part of its mission which is to support children with cancer, reduce the child mortality rate and create an appropriate environment that empowers families who have children with cancer. Pediatric stem cell transplant also is used to treat many types of conditions affecting children and adolescent, including cancer and certain hematologic, immunologic and genetic disorders. The pediatric stem cell transplantation ward was inaugurated in Mahak hospital (Iran-Tehran) on April, 2012. Pediatric stem cell transplant ward practice that performs about 34 transplants per year. All patients are kept in high efficiency particulate air (HEPA)-filtered, positive-air-pressure-sealed rooms, and strict hand hygiene is practiced. We analyzed the outcome of 145 patients from a single institution who underwent allogeneic & autologous stem cell transplantation from between 2012 and 2016. 130 of patients had peripheral blood stem cell as the stem cell source, 14 of patients' bone marrow and in 1 patient cord blood used. The majority of patients are: ALL = 42, neuroblastoma = 31, AML = 17, Hodgkin's dis = 30, retinoblastoma = 4, Ewing's sarcoma = 2, rhabdomyosarcom = 2, Wilm's tumor = 4, hepatoblastoma = 1, aplastic anemia = 3, hemoglobinopathy = 3, germ cell tumor = 4, epedymoma = 1, osteopetrosis = 1. The conditioning regimens used were mainly myeloablative in allogeneic transplantation (BUCY4). Age of patients 7 month to 26 years with median age 9 years, M/F = 83/62. 64 patients transplanted Allo HSCT and 81 patients transplanted Auto HSCT. GVHD prophylaxis regimen was cyclosporine + Mtx in Allo HSCT. All patients engrafted. The type of donor in allogeneic SCT includes 55 related sibling and 9 unrelated allogeneic. In allogeneic PBST patients' median time to reach absolute neutrophil count (ANC)  $>0.5 \times 10^9/L$  was 11 days, and the median time to platelet count  $>20 \times 10^9/L$  was 13 days vs 19 and 22 days in Allo BM patients. In autologous PBST median time to reach absolute neutrophil count  $>0.5 \times 10^9/L$  was 13.5 days, and the median time to

platelet count  $>20 \times 10^9$  was 17 days (81 pt's). Acute graft-versus-host disease of grade II to IV was observed in 67% of patients and chronic graft-versus-host disease in 59% of patients. At present 124 pt's are alive (88%) and 21 pt's died due to ARDS, VOD, hemorrhagic stroke, sepsis and relapse. With a median follow-up of 35 months (2–51 months) after transplant the four years overall survival were 81.1% and event-free survival was 80.1%. In Hodgkin's disease patient's overall survival and event free survival after autologous SCT better than neuroblastoma. Autologous SCT can lead to durable remissions in children and adolescents with Hodgkin's disease & solid tumor. These results indicate that despite our ward is new status both allogeneic and autologous HSCT are feasible with outcomes similar to developed countries. These preliminary data suggest that HSCTs have been used as one of the standard treatments for hematological diseases and malignancies in Iran.

**Disclosure of conflict of interest:** None.

#### P467

##### Hematopoietic stem cell transplantation of pediatric chronic myeloid leukemia in first chronic phase: a multicenter study by the Turkish pediatric bone marrow transplantation study group (TPBMT-SG)

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Chronic myeloid leukemia (CML) is a rare disorder in children. They have longer life expectancies than adults; the goal of CML treatment in children should be cure rather than disease suppression. Because of the possibility of decades-long tyrosine kinase inhibitor (TKI) treatment, which also occurs during periods of active growth, morbidity related to TKI therapy for CML is different in children than in adults. On top of all these, the role of hematopoietic stem cell transplantation (SCT) is still not clear in children with CML especially in first chronic phase (CML-CP). The aim of the study was to determine the outcome (mortality, relapse and long term survival) of children with CML-CP who received allogeneic stem cell transplantation in Turkey. 70 children were transplanted for CML in Turkey between January 2000 and December 2015. SCT was performed in first chronic phase in 47 (67 %) of the patients. We retrospectively analyzed the data of Turkish Pediatric BMT registry for CML-CP who received allogeneic SCT at 8 pediatric centers in Turkey. Twenty-three boys and 24 girls with CML-CP whose ages ranged between 2–17 years (median 13.5 years, 19% < 10 years). The median time to transplant from the diagnosis was 12 months (range: 3–66 months). TKIs were used in 90 % of the patients for median 11 months (range: 1–63 months) and complete or major molecular response was detected 41% of all patients prior to SCT. The donor was HLA matched related (MRD) in 30 (64%) (26 matched sibling (MSD), 4 related family donor), matched unrelated (MUD) in 16 (34%) and mismatched related in 1 (2%) patients. Busulphan based chemotherapy were used for conditioning (+ cyclophosphamide [79%] or fludarabine [21%]) in all patients. Graft source was bone marrow in 33 (70%), peripheral stem cells in 13 (28%) and combined in 1 (2%) patient. Graft versus host disease (GVHD) prophylaxis was with cyclosporine alone or combined in all patients. Two patients underwent second transplantation from the same donor because of engraftment failure. Grade III/IV acute and extensive chronic GVHD were seen 15% for both. The median

follow up time was 45 months for patients and complete molecular remission was obtained in all survived patients. The 5 year estimate of overall survival (OS) and event free survival (EFS) for the patients was 78 % and 75 % respectively. EFS was 79% and 72% when the donor was MSD or MUD ( $P=0.65$ ). Transplant related mortality was 17%. Disease relapsed clinically in 3 patients, molecular relapse was observed in 5 patients. Ovarian insufficiency was observed in a female patient. Molecular failure to imatinib treatment was found 42% of the patients with CML-CP in another pediatric study in Turkey. SCT is a curative therapy for pediatric CML patients especially in early phase of disease. Transplant related mortality is higher than desirable and long term toxicities are required for our patients. The recent development of reduced-toxicity BMT, particularly identical sibling SCT, may play a bigger role in the treatment of children with CML in the future. There is a need for standardization of the SCT procedure and also we need more information about the curability and morbidity of TKIs.

**Disclosure of conflict of interest:** None.

#### P468

### High overall concordance, but discordant absolute level of MRD by flow cytometry and real-time PCR in pediatric ALL

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MRD monitoring pre and post-HSCT for paediatric ALL has a strong prognostic significance and can guide early intervention after paediatric HSCT, e.g. withdrawal of immunosuppressive agents, donor lymphocyte infusions or targeted anti-leukemic therapy. Multiparameter flow cytometry (FCM) and IG/TCR real-time quantitative PCR (PCR) are the primary applied methods to detect MRD in ALL.<sup>1,2</sup> We included children < 18 years of age, transplanted for ALL at the national paediatric transplant centre in Copenhagen between 2008 and 2015, that had samples analysed concurrently for MRD by PCR and FCM. Data from pre and/or early post-HSCT bone marrow samples was collected. Early post-HSCT MRD was defined as within 30 to 200 days after HSCT. According to the NOPHO ALL-2008 protocol, BCP-ALL was stratified mainly based on MRD flow cytometry, and for T-ALL based on MDR-PCR assessment of primary samples if informative markers were available. In the study period, 42 transplantations were performed on children with ALL. Of these, data from 30 transplantations in 28 children was available for comparative analysis, comprising 51 parallel MRD analyses. Median (range) age at SCT was 8 (1–18) years, and median follow up time was 13 (2–72) months. HSCT was performed in 1<sup>st</sup> complete remission (CR) in 17 (54%), 2<sup>nd</sup> CR in 9 (32%), and 3<sup>rd</sup> CR in 3 (8%). A total of 23 (82%) were diagnosed with B-cell ALL, 4 (14%) with T-cell ALL and one (4%) with unspecified ALL. In total, 5 (17%) children relapsed, a median of 3 (2–35) months from SCT. In total, 32 (63%) samples were negative by both methods, 8 (16%) were positive by both methods, 7 (14%) were negative by PCR but positive by FCM and 1 (2%) was positive by PCR but negative by FCM. Quantitative MRD values with PCR and FCM were highly correlated when all samples were evaluated (Spearman's rho = 0.62,  $P < 0.00001$ ) (Figure 1). However, of the 8 samples that were double positive, the values did not correlate (Spearman's rho = 0.54,  $P = 0.1702$ ). We show a high concordance between MRD analysis with PCR and FCM overall, but no quantitative correlation in those samples with positive MRD by both methods. Further comparison of predictive value of the two methods is warranted.

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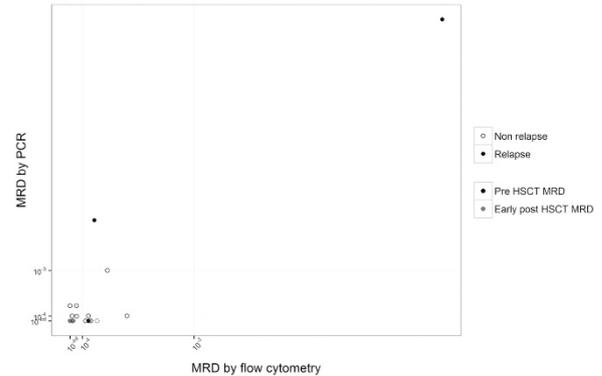
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[P468]

Figure 1. MRD measurements by flow cytometry and PCR.



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#### P469

### Immune reconstitution inflammatory syndrome in a SCID patient after DLI

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Immune reconstitution inflammatory syndrome (IRIS) is a clinical condition emerging after immune recovery of an immunocompromised status, mostly after the initiation antiretroviral therapy (ART) in human immunodeficiency virus (HIV) infected patients, but also in several other settings, such as the recovery from the severe combined immunodeficiency (SCID) status after hematopoietic stem cell transplantation (HSCT). Herein, we report a patient transplanted for SCID who developed IRIS for two times, namely shortly after transplantation and after donor lymphocyte infusion (DLI) (Table 1) In our patient, T cells passing from the donor probably contributed to the immediate post-transplant increase in the size of granulomas. This inflammatory response waned after the institution of immunosuppressive and methylprednisolone therapy. However, immunosuppressives were stopped due to lowered chimerism at follow-up, and the inflammatory response re-appeared after administering stem cell support containing a large amount of T cell from the donor for DLI purpose. Although the mechanism by which DLI results in clinical responses is unclear, it is presumed to be a T cell-mediated process. Several studies have been performed to strengthen our understanding of the immunopathogenesis of IRIS. While some of those studies put forth T cell-associated causes, others implicated cytokines and non-T cells. The reaction that developed in our patient is suggestive of T cell-associated causes. Immune reconstitution inflammatory syndrome remains a poorly understood entity. The DLI procedure in our case provides a unique clue supporting a T cell mediated process. Pediatric transplant teams need to be

aware of the previous IRIS phenomenon of BCG-adenitis while making the decision of DLIs.  
[P469]

Table 1: The clinical and laboratory data

Posttransplant	Chimerism	ALC	Cd3 %	Cd19 %	IgG	Follow-up
20					487	Enlargement of granulomas
28	100					Tapering of steroid
35						IRIS, restart of steroids
60						Shrinkage of granulomas, tapering steroids
86	60				743	Decreased chimerism, disc. of CsA
107	44	520				DLI
126	29	340			443	Enlargement of granulomas
139						
175	49	820	75	6	387	
212					2588	
258						Healing lesions with crusts
280	25				1908	
349		1160	73	2	1531	
440	34	720	66	3	898	General Vaccination
560	20	720			965	Fibrotic lesions
897	35	650	65	6	641	
1081	18	830	58	8	894	
1275	36	870			841	

**Disclosure of conflict of interest:** None.

**P470**  
**Impact of hematopoietic stem cell transplantation on dental development**

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Pediatric patients treated with a hematopoietic stem cell transplantation (HSCT) often suffer from late side effects caused by the treatment. The aim of this study is to investigate the late effects of a HSCT on dental development. In addition, patients and parents awareness on this topic was investigated. 42 young adults treated and followed at the Ghent University Hospital who were under the age of 12 y at the time of HSCT were examined clinically and radiographically (Planmeca ProMax 2D). Transplants (11 autologous/31 allogeneic) were done for malignant disease in 34 pts. Eight patients received a HSCT for a non-malignant disease. Twelve patients underwent a conditioning regimen with total body irradiation (TBI), 21 patients with busulfan and 9 patients with other chemotherapeutic agents. 16 patients were < 3 y, 9 patients were 3–6 years and 17 patients were > 6 years at HSCT. Every patient was evaluated on dental agenesis, microdontia and root-crown ratio. Patients and their parents were asked about their knowledge and interest for dental screening at the follow-up clinic using a questionnaire. Overall, the prevalence of agenesis and microdontia of one or more dental elements is respectively 51.3% and 46.2% in our study population. 76.3% of patients have a strongly aberrant root-crown ratio of at least one element. Patients treated < 3 years of age show significantly more microdontia (76.9%;  $P < 0.001$ ) as well as agenesis (92.3%;  $P < 0.001$ ) compared to patients treated at an older age. The first premolar of the mandible is the most vulnerable element for agenesis as well as for microdontia. More microdontia is found in patients treated with a busulfan conditioning regimen compared to the other conditioning

regimens (68.4% versus 25%). Patients older than 6 years, treated with busulfan have statistically more microdontia compared to patients > 6 y treated with TBI conditioning regimen ( $P = 0.044$ ). There was no difference of the conditioning regimens on agenesis nor on root-crown ratio. Almost all patients/parents find it important to receive information about the dental late effects of a HSCT and are interested in dental screening at the follow-up clinic. Treatment with HSCT has an explicit negative impact on dental development. The degree of this effect depends on age at HSCT and used conditioning-regimen. Dental follow-up of these patients is essential and should be incorporated in the follow-up program.

**Disclosure of conflict of interest:** None.

**P471**  
**Importance of body composition in the outcome of hematopoietic stem cell transplantation in elderly patients**

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The loss of muscular mass is a well recognized cause of the decline in muscle strength and functionality that accompany the aging process. In 1989, Irwin Rosenberg proposed the term 'sarcopenia' to describe the decline in muscle mass associated with aging. Changes in body composition after Hematopoietic Stem Cell Transplantation (HSCT) have been the subject of previous studies. Immunosuppressive therapy and corticosteroids are known to alter skeletal muscle metabolism. Infections and graft-versus-host disease (GVHD) that can occur after HSCT may also affect body weight and composition. Therefore, both the treatment and complications after HSCT exert large negative effects on lean muscle mass, especially in elderly patients. Patients with hematologic malignancies are usually well nourished before undergoing HSCT. Objective: The aim of this study is to determine in an elderly population whether parameters of body composition could be correlated to outcomes after HSCT. We performed a retrospective longitudinal study through review of medical records of 48 patients ≥ 60 years old undergoing HSCT at Hospital Israelita Albert

Einstein, from 2013 to 2015, that were subject to tomography scans (CTs) in a period ranging from 60 days before and 15 days after HSCT. Body composition data were analyzed in CTs in T4 level by Sliceomatic program. Descriptive statistics were calculated by SSPS program with the following parameters: age, body mass index, hand grip and corporal composition parameters. Of the 48 patients evaluated, 24 were male. The median age was 67 years ( $\pm 4.2$ ). The underlying diseases and respective percentages in this cohort of patients were: multiple myeloma: 35.4%; myelodysplastic syndrome: 18.8%, lymphoma; 14.6%, acute myeloid leukemia: 10.4%. Patients with diagnosis of amyloidosis, cutaneous lymphoma and lymphocytic lymphoma comprised 20.8 % of the patients. Regarding the type of HSCT, 50% were autologous, 45.8% were allogeneic and 4.2% were haploidentical. In relation to body mass index (BMI), 45.8% of patients were in the normal range, 21% were overweight, 5% underweight and 28% were not analyzed. CTs evaluation found an average muscle area of 151cm<sup>2</sup> ( $\pm 41$ ) and subcutaneous adipose tissue of 230.5 cm<sup>2</sup> ( $\pm 78$ ). The Hand Grip median was 29 kgf ( $\pm 9.2$ ). Of the 48 patients evaluated, neutrophil engraftment had a median of 13 days ( $\pm 4.3$ ). Seventeen patients developed acute GVHD; of these 9 had grade I-II and 8 had grade III-IV. Of the whole cohort 60.4% of patients are alive. Among the 19 deaths, 10 were not related to relapse. Among the tested parameters the only positive correlation was found between neutrophil engraftment and subcutaneous adipose tissue ( $r=0.8$ ,  $P<0.05$ ). Evaluation of the body composition in patients to be submitted to HSTC may offer prognosis-associated parameters important to a better nutritional and geriatric management.

**Disclosure of conflict of interest:** None.

#### P472

### Strategies to reduce neutropenic fever and hospital readmission in lymphoma and multiple myeloma patients managed at-home after autologous stem cell transplantation

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Readmission due to neutropenic fever (NF) is not infrequent in an at home-care autologous stem cell transplantation (ASCT) program. We implemented two strategies to reduce it: withdrawal of G-CSF, with the intention to decrease engraft-

ment syndrome, and intensification of antibiotic prophylaxis. Between November 2000 and September 2015, 219 patients were managed at-home since day +1 of ASCT. Lymphoma (Ly) patients ( $n=136$ ) were conditioned with BEAM and multiple myeloma (MM) patients ( $n=83$ ) with standard dose of melphalan. All patients received prophylaxis with quinolone, fluconazole, aerosolized pentamidine and low-dose acyclovir if herpes serology was positive. In Ly patients, 61 received prophylactic ceftriaxone (Ct) 1 g/day i.v. (group Ly-Ct) and 75 piperacillin-tazobactam (PT) 4.5 g/8 h i.v. using a portable intermittent infusion pump (group Ly-PT). In all MM patients prophylactic Ct was used; 32 received G-CSF (5 mcg/kg/day) (group MM-G) starting on day +7 until granulocyte recovery, and 51 did not receive G-CSF (group MM-noG). First-line therapy at home of NF was PT 4.5 g/6 h i.v. (if prophylactic Ct) or refrigerated meropenem 1 g/8 h i.v. (if prophylactic PT), using a portable intermittent infusion pump for both drugs. Fever was an indication of immediate visit in the hospital, and those patients presenting with focal infection or signs of severe sepsis were admitted. Other indications for readmission were: willingness of the patient or caregiver; uncontrolled nausea, vomiting or diarrhoea and mucositis requiring total parenteral nutrition or i.v. morphics. The main characteristics of the patients and outcomes are shown in Table 1. There were no differences between groups with respect to age, gender, diagnosis, stage of disease, and source of stem cells. In Ly patients, the quantity of peripheral CD34+ cell dose ( $\times 10^6$ /kg) infused was different between groups (group Ly-Ct: 3.4 (1.5–21.6) and group Ly-PT: 5.7 (1.6–17.6);  $P<0.001$ ), but duration of neutropenia was similar. The incidence of grades  $\geq 2$  mucositis (NCI-CTC-score) was significantly higher in group Ly-Ct (37.7% vs 16%,  $P=0.004$ ), as well as fever (83.6% vs 45.3%;  $P<0.001$ ), with the start earlier in group Ly-Ct (+5 (2–10) vs +6 (2–10);  $P=0.044$ ). Bacterial infection (Ly-Ct vs Ly-PT) was documented in 16.4% vs 5.3% ( $P=0.035$ ), and coagulase-negative *Staphylococci* was the more frequent bacteria isolated. Readmission was required in 9 (14.8%) patients in group Ly-Ct and 1 (1.3%) in Ly-PT ( $P=0.008$ ). Regarding MM patients, recovery (days) of granulocyte count above  $0.5 \times 10^9$ /L was faster in group MM-G (8 (5–22) vs 10 (6–18);  $P=0.01$ ). The incidence of NF was significantly higher in group MM-G (19 (59.4%) vs 19 (37.3%);  $P=0.049$ ). No differences were observed in the incidence and severity of mucositis, first day and duration of fever, documented bacterial infections or readmission rate between MM patients groups. This study suggests that in at home ASCT, the use of piperacillin-tazobactam prophylaxis significantly reduces the incidence of neutropenic fever and hospital readmission in patients with Ly, and also that no administration of G-CSF in MM patients reduces significantly the incidence of neutropenic fever.

**Disclosure of conflict of interest:** None.

[P472]

Table 1. Main characteristics and outcomes

Characteristics	Total group (n=219)	Lymphoma (n=136)			Multiple myeloma (n=83)		
		Ly-Ct (n=61)	Ly-PT (n=75)	P	MM-G (n=32)	MM-noG (n=51)	P
Age *	49 (17-71)	41.9 (17-67)	45.6 (19-71)	NS	51.75 (25-67)	55.25 (40-69)	NS
Gender (F/M)	78/141	19/42	30/45	NS	12/20	17/34	NS
CD34+ *	3.6 (1.5-21.6)	3.4 (1.5-21.6)	5.7 (1.6-17.6)	<0.001	3.2 (1.9-6.4)	3.2 (1.9-9.4)	NS
Days of neutrophils $\leq 0.5 \times 10^9/L$ *	9 (5-22)	9 (6-19)	8 (6-14)	0.052	8 (5-22)	10 (6-18)	0.01
WHO grade II-IV mucositis **	37 (16.9)	23 (37.7)	75 (16)	0.004	2 (6.3)	0 (0)	NS
Neutropenic fever ( $\geq 38^\circ C$ ) **	123 (56.2)	51 (83.6)	34 (45.3)	<0.001	19 (59.4)	19 (37.3)	0.049
First day with fever *	6 (2-12)	5 (2-10)	6 (2-10)	0.044	7.5 (4-12)	8 (3-11)	NS
Duration of fever *	2 (1-11)	2 (1-11)	2 (1-6)	0.045	2 (1-5)	1 (1-5)	NS
Positive blood cultures **	19 (8.7)	10 (16.4)	4 (5.3)	0.035	2 (2.4)	3 (5.9)	NS
Readmissions **	16 (7.3)	9 (14.8)	1 (1.3)	0.008	4 (12.5)	2 (3.9)	NS

CR: complete remission; NS: not significant.

\*range, \*\*%.

**P473****Is haploidentical transplantation a salvage therapy for pediatric patients requiring urgent transplant?**

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Stem cell transplantation (SCT) is a well established procedure for patients with malignant and non-malignant diseases, but it is limited by the availability of related or unrelated compatible donors. The new methods to control the alloreactivity—especially the use of posttransplant cyclophosphamide (PTCY) in the haplotransplant field—expanded the donor pool and allowed the access to the SCT for more patients. This approach is very attractive for patients requiring an urgent transplant.

**Aim:** We present the first year experience with haploidentical SCT in children with malignant diseases in Fundeni Clinical Institute, Bucharest, Romania. The haploidentical program for children started in 2016 in Fundeni Clinical Institute and 2 patients were included: high risk AML, primary graft failure after unrelated 9/10 SCT (1 case), juvenile myelomonocytic leukemia (JMML) without compatible donors (1 case). The criteria for haploidentical SCT were: (a) emergency, (b) no available donor 9-10/10, (c) no alternative therapy, (d) informed consent signed by parents. The conditioning regimen included Fludarabine 150 mg/m<sup>2</sup> and Melphalan 140 mg/m<sup>2</sup>. The choice of parental donor was decided after analysis of anti HLA antibodies presence. Both patients received peripheral stem cells from fathers. The G-CSF administration started from day +12. The GVHD prophylaxis consisted in PTCY 50 mg/kg/day, day +3, +4 plus tacrolimus and MMF from day +5. Case 1: 8y old boy with high risk AML, non-engrafted after BU-Cy conditioning and unrelated 9/10 donor transplantation with major ABO incompatibility received (on day +55 after first SCT) the second SCT, with 11 × 10<sup>6</sup> CD 34/kg peripheral stem cells from his father, with 6/10 compatibility. We recorded the PMN engraftment on day +16 and the platelet engraftment on day +24. Complete donor chimerism (100%) is maintained from day +16 until present (11 months). No infections were recorded. The patient presented only grade I skin GVHD. In the absence of GvHD, MMF was stopped on day +30, tacrolimus tapered from day +60. the last follow-up showed very good clinical condition, complete remission, no GVHD, without immunosuppression. Case 2: 18 mo old boy with JMML, in transformation received 8/10 compatible peripheral stem cells, 8 × 10<sup>6</sup> CD34/kg from his father. The PMN engrafted on day +17 and platelets engrafted on day +30. The chimerism analysis showed mixed chimerism on day +19, with 48% donor cell origin. Tacrolimus and MMF were progressively reduced, but due to progressively mixed chimerism, the DLI 1 × 10<sup>5</sup> CD3/Kg was infused on day +26. Patient developed grade IV skin and gut GVHD and started corticosteroids with favorable response. CMV pneumonia developed on day +125. The close chimerism monitoring showed progressive mixed chimera, with the lowest value of 5% donor origin. All tentative to decrease immunosuppression determined a GVHD rebound. For a better JMML control, low doses of 6 MP and Ara C were recommended. Last follow-up (10 mo after SCT) showed good clinical condition, chronic skin and gut GVHD, no splenomegaly, monocytes 2.3 × 10<sup>9</sup>/L, normal hemoglobin, platelets 40 × 10<sup>9</sup>/L and mixed chimerism 18% donor origin. Haploidentical SCT appear to be a safe alternative for pediatric patients requiring salvage transplant procedures.

**Disclosure of conflict of interest:** None.

**P474****KIR RL mismatch leads to enhanced NK activation, cytolysis and ADCC in B-lineage precursor ALL**

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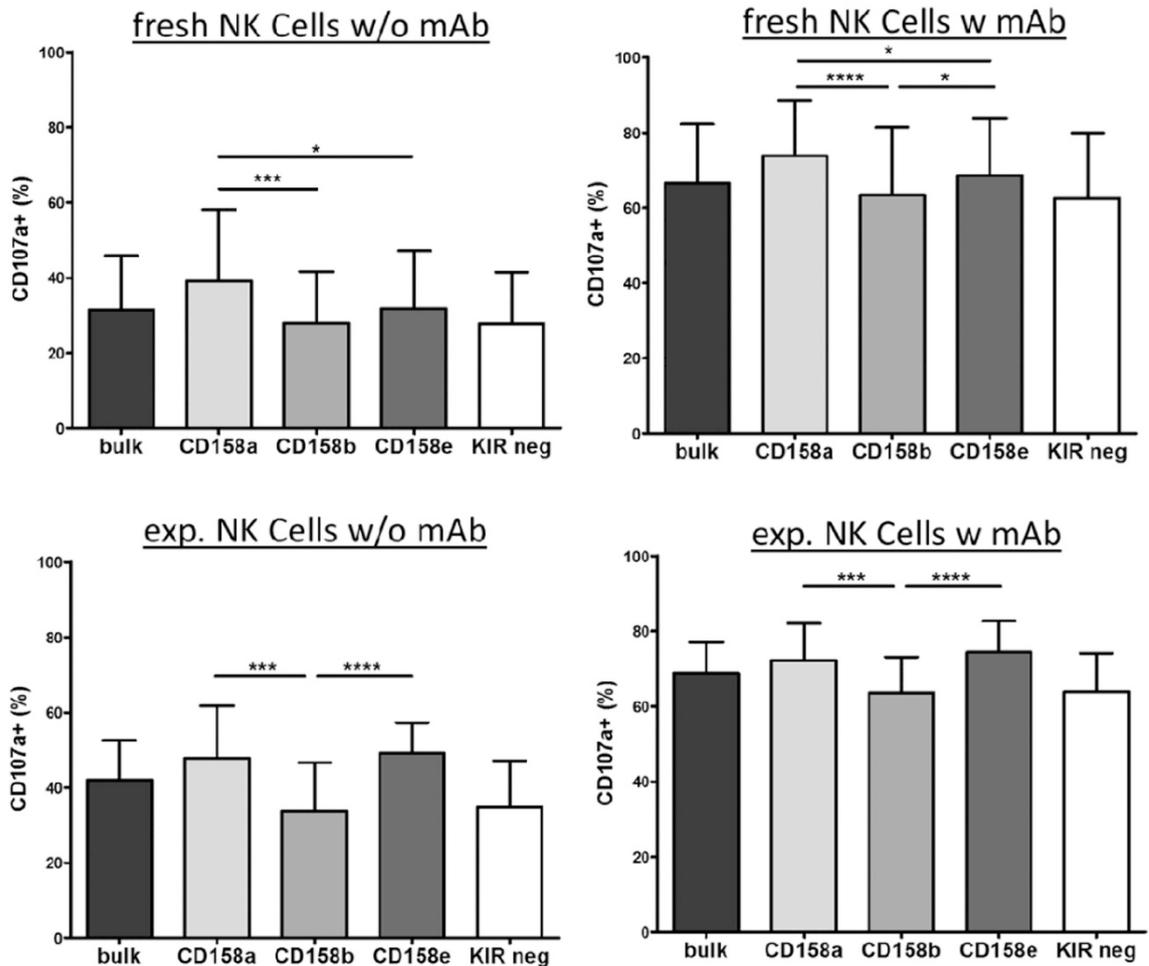
KIR/HLA interaction has been shown absolutely relevant for the survival of patients suffering from leukemia.<sup>1</sup> This was shown in childhood ALL after haploidentical SCT.<sup>2</sup> Fc modified antibodies can enhance ADCC efficiently irrespective of KIR geno- and phenotype and FcγIIa polymorphism<sup>3</sup> and can reduce MRD in the context of HSCT.<sup>4</sup> NK cells from healthy donors (*n* = 12) were characterized by KIR genotyping and NK phenotyping (KIR, NKG2D, DNAM-1, NKp46). CD107a assay, intracellular IFNγ staining and cytotoxicity assays using primary PBMCs as well as preactivated and expanded NK and γδ T cells with and without the anti-CD19 mAb 4G7 with SDIE-modification were used to elucidate the impact of KIR RL interaction on ADCC in BCP-ALL. Medium antileukemic activity of primary NK cells was observed. Expansion of NK cells significantly increased activation (CD107a), cytokine production (IFNγ, TNFα) and cytolysis (*n* = 12, *P* < 0.001). Moreover, the 4G7SDIE mAb significantly increased the activation and cytolysis by NK cells and γδ T cells in primary as well as in expanded effector cells (*n* = 12, *P* < 0.001). Qualitative (HLA-I group missing) and also functional RL-mismatch (low expression of corresponding HLA-I group) showed a significant impact on NK degranulation (*n* = 12, *P* < 0.01 in all conditions). Upregulation of HLA-I on BCP-ALL as well as blocking experiments using w6/32 mAb clearly demonstrated the relevance of KIR-R-HLA-I interaction for NK mediated recognition and cytolysis. NK degranulation positively correlated with activatory NK cell receptors (NKG2D, DNAM-1 and NKp46). Irrespective of the BCP-ALL cell line, superior and inferior donors were identified by in vitro testing. Receptor-ligand KIR mismatch determines recognition, cytolysis and ADCC in BCP-ALL by primary, preactivated and expanded NK and γδ T cells. Especially KIR2DL1 RL mismatch seems to exert strong NK alloreactivity. Functional effector cell assessment in addition to KIR-genotyping might help to identify most superior donors in haploidentical HSCT.

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[P474]

## Differences between KIR-subgroups



Disclosure of conflict of interest: None.

P475

### Lomustine-containing conditioning regimen CEM (lomustine, etoposide, melphalan) in autologous stem cell transplant (ASCT) for children with relapsed/refractory lymphomas (single center experience)

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[P475]

Table 1. Conditioning regimen CEM

	D-4	D-3	D-2	D-1	D0
Lomustine(CCNU) po 300 (400) mg/m <sup>2</sup>	X				
Etoposide 1200 mg/m <sup>2</sup>		X			
Melphalan 180 mg/m <sup>2</sup>			X		
Autologous SCT					X

Most of the modern conditioning regimen used for ASCT in children with relapsed/refractory lymphomas include Carmustine known as high pulmonary toxicity drug. Additionally it's not present in pharmaceutical market of Ukraine. The same time there are some reports about Lomustine-containing conditioning regimen for allo- and auto-SCT in adults. Our center designed CCNU-containing conditioning regimen (CEM), see. Tab.1, and regular use it since 2005. In this report we present a 11-year results of ASCT with CEM conditioning regimen in children with relapsed/refractory lymphoma. Single center prospective observational study: 48 pediatric pts with relapsed/refractory lymphoma; aged 6-17 y (median=15

y); m=27, f=21. Diagnoses: Hodgkin's lymphoma(HL)- 38 pts (refractory 22; relapsed 16); non-Hodgkin's lymphoma(NHL) - 10 pts (refractory 9; relapsed 1). Disease status before ASCT: 1st (after refractory)-2ndCR: 18/48 (HL-13/38; NHL-5/10); PR: 28/48 (HL-24/38; NHL-4/10); SD: 2/48 (HL-1/38; NHL-1/10). Notable features: Primary lung involvement - 18/48 (37.5%); Prior radiotherapy to the mediastinum - 19/48 (39.6%); Heavily pretreated patients with advanced disease ( $\geq 3$  lines previous treatment) 18/48 (37.5%). Grafts: PBSC - 43/48 pts with median of CD34+cells- 3.5x106/kg (1.7÷16.4); 2 - poor grafts CD34+ $\leq 2 \times 106$ /kg; PBSC+BM - 4 pts; BM - 1 pt. CCNU dose: 47/48 pts 300mg/m<sup>2</sup>; 1pt 400 mg/m<sup>2</sup>. Engraftment: ANC>500 cells/mkl: median=D+11(8÷24), 47/48pts. PLT>50 000 cells/mkl: median=D+ 28(13÷122), 43/48pts. Full engrafted 43/48pts. Pulmonary toxicities: pneumonitis 8/48 pts (D+ 8÷39), 1/8 pt required a short-term mechanical ventilation (2 of them died because of lung infection ad D+68 and D+82). Follow-up (07.12.16): median = 55 months (4 - 132 months). 38/48 pts alive (35/48 pts-72.9%-in CR with duration 1-132 mo); TRM - 4 pts (8.3%): 1pt-D+9 (viral hepatitis B+G reactivation);1pt-D+35 (Ps.Aeruginosa associated sepsis on a background of graft failure); 2pts (4.2%)-D+68 and D+82 (pulmonary toxicities +infection; both had prior mediastinal radiotherapy). Relapse/progression after ASCT- 8/48 pts (16.6%), 6 of them died. 1 pt achieved secondary MDS (diagnosed 4.5 mo after ASCT). For this group of pts with relapsed/refractory lymphomas (n=48) 11-year OS=0.76 (SE±0.08); for HL(n=38) EFS = 0.73 (SE ± 0.09); for NHL(n=10) EFS=0.88 (SE ± 0.2) Lomustine-containing conditioning regimen CEM (Lomustine, Etoposide, Melphalan) is effective and feasible in autologous stem cell transplant (ASCT) for children with relapsed/refractory lymphomas. Acute pulmonary toxicity is acceptable even in heavily pretreated patients.

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#### P476

##### Long-term brain status and cognitive functioning in children treated for high-risk acute lymphoblastic leukemia with and without allogeneic hematopoietic stem cell transplantation

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Central nervous system (CNS) damage is one of the most clinically relevant complications after acute lymphoblastic leukemia (ALL) treatment in children, especially those who got CNS irradiation. The aim of the study was to assess long-term consequences of CNS prophylaxis in patients with high risk ALL (HR-ALL) treated according to ALL IC-BFM 2002 and to compare observed abnormalities in patients who underwent allogeneic hematopoietic cell transplantation (allo-HSCT) to those HR-ALL patients who received only prophylactic CNS irradiation (12 Gy) and to control group. We studied 46 children aged 7.4–18.6 years (median: 11.5), including 30 children cured from HR-ALL (16 with allo-HSCT and 14 without HSCT) and 16 children with newly diagnosed ALL (control

group). In studied group median time from therapy completed was 62 months. All patients were treated according to the HR arm of ALL IC-BFM 2002 (16 with allo-HSCT and 14 without HSCT). In HSCT group 14 patients were conditioned with FTBI (12 Gy) and 2 with high-dose chemotherapy alone. To assess brain status, volumetric T1 and T2-weighted sequences of magnetic resonance imaging were used, which allowed for segmentation and volumetric measurements of subcortical structures (hippocampus, thalamus, amygdala, putamen, globus pallidus, caudate nucleus). Neuropsychological assessment based on battery neuropsychological tests, i.e. Wechsler Intelligence Test for Children (WISC-R; IQ assessment and long-term memory), Rey Auditory Verbal Learning Test (RAVLT; evaluation of short term memory), Benton Visual Retention Test (BVRT; evaluation of visual-spatial memory), Verbal Fluency Test (evaluation of verbal memory), the Stroop test (ST; evaluation of reaction time and executive) and Wisconsin Card Sorting Test (WSCT; evaluation of executive functions). All studied patients had significantly lower volume of the amygdala in comparison to control group ( $P=0.048$  i  $P=0.045$ , respectively). Volumes of other subcortical structures did not differ significantly between compared groups. Patients undergoing allo-HSCT had lower average IQ level in both, verbal scale ( $P=0.033$  i  $P=0.022$ ) and full scale ( $P=0.011$  i  $P=0.044$ ) in WISC-R, worse visual-spatial memory ( $P=0.021$  i  $P=0.011$ ) in BVRT in comparison to HR-ALL patients treated without allo-HSCT and control group. Moreover, transplanted patients performed significantly worse on measures of executive functioning ( $P=0.024$  i  $P=0.018$ ) in WSCT. They had also slower processing speed ( $P=0.008$ ) in ST when compared to the control group, but with no significant difference to non-transplanted HR-ALL patients. There was no differences in short term memory and verbal memory between groups. (1) All patients treated for HR-ALL, both transplanted and non-transplanted, had reduced amygdala volume. (2) Only transplanted patients had cognitive impairment in IQ, visual-spatial memory, processing speed and executive functions domains that were not observed in non-transplanted patients.

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#### P477

##### Long-term outcome and chimerism in patients with Wiskott–Aldrich syndrome treated by haematopoietic cell transplantation: TRUMP data analysis in a collaborative study of The Japan society for haematopoietic cell transplantation

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Allogeneic stem cell transplantation (SCT) has been recognized as a curative treatment for patients with Wiskott–Aldrich syndrome (WAS). In SCT for WAS, myeloablative conditioning (MAC) has been indicated to avoid a mixed chimera. However, risk factors for a mixed chimera in patients with WAS who have received SCT have not been evaluated. Here, we

analyzed the outcomes of SCT and risk factors for a mixed chimera in 108 patients with WAS who underwent SCT in Japan since 1985. We reviewed medical records of 108 consecutive WAS patients who received SCT since January 1985 who were registered with The Japan Society for Hematopoietic Cell Transplantation. The age of the patients at transplantation ranged from 3 months to 23 years, and the mean age was 3.81 years. The origin of the stem cells was related bone marrow (BM) or peripheral blood stem cells (PBSC), unrelated BM or PBSC, and unrelated cord blood (CB) for 36, 41 and 27 patients, respectively. A preparative conditioning regimen consisting of MAC was provided to 76 patients, and reduced-intensity conditioning was provided to 30 patients. Fifty-one patients received prophylaxis against graft-versus-host disease (GVHD) with cyclosporine in combination with methotrexate (MTX) or a steroid, and 51 patients received tacrolimus (Tac) with MTX or a steroid. Chimerism analysis had been performed in 91 patients. Neutrophil engraftment was achieved in 91 patients (82.7%). The engraftment rate was significantly higher in patients who received Tac for GVHD prophylaxis, ( $P=0.0001$ ) Overall survival rate was significantly higher in patients with complete chimerism than in patients with mixed chimerism ( $88.2\pm 6.1\%$  and  $66.7\pm 9.9\%$ , respectively,  $P=0.003$ ), though there was no significant difference in stem cell sources. Using multivariate analysis, the rate of complete chimerism in patients who received MAC including cyclophosphamide (CY) at more than 200 mg/kg was significantly higher ( $P=0.02$ ) than the other conditioning. Not only patients with mixed chimerism but also patients with complete chimerism were complicated with auto-immune diseases. In this study, achievement of complete chimerism after SCT was important for survival in patients with WAS. We found that patients who underwent MAC including CY at more than 200 mg/kg had a higher rate of complete chimerism. We also found a higher neutrophil engraftment rate in patients who received Tac for GVHD prophylaxis. Thus, MAC including CY at more than 200 mg/kg and TAC for GVHD prophylaxis are optimal conditions of SCT for patients with WAS.

**Disclosure of conflict of interest:** None.

#### P478

##### Maternal T-cell engraftment: a first case in an adenosine deaminase (ADA) deficient child

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Adenosine Deaminase (ADA) deficiency is an inherited autosomal recessive immunodeficiency which represents about 10–15% of SCID. Since 1992 we diagnosed 29 patients affected by ADA-SCID: 10 underwent Hematopoietic Stem Cell Transplantation (HSCT), 10 were treated with replacement therapy with PEG-ADA and 4 received gene therapy; 5 patients died before or after treatment. Maternal T lymphocyte engraftment is frequently detected in SCID patients, but this is never been found in ADA deficient patients. A 3-months-old Italian girl, from non-consanguineous parents, presented to our hospital with a history of frequent bronchiolitis associated with dermatitis, mycosis, hypogammaglobulinaemia, marked lymphopenia (T cells CD3, 171/mmc; CD3/CD4, 158/mmc; CD3/CD8, 8/mmc, B cells 15.2/mmc, and NK cells, 110/mmc) and in vitro absence of proliferative response to PHA. Level of immunoglobulins was almost normal (IgG 439 mg/dl, IgA 87 mg/dl, IgM 57 mg/dl). High levels of toxic metabolites were found: AXP, 1.573 micromol/ml RBC; dAXP, 0.629 micromol/ml

RBC; %dAXP, 28.5. ADA activity in RBC lysates was abnormally high for SCID-ADA (0.54 U/g Hb). Molecular analysis confirmed diagnosis: the sequencing of exon 10 revealed two mutations: a missense mutation previously reported called p.Ser291Leu (c. 872C>T) and a new missense mutation defined p. Leu298Pro (c.893T>C). T-cells STR analysis of patient showed 54.1% maternal T lymphocytes engraftment never reported before in ADA-SCID patients. The girl was transferred to the isolated BMT unit and the respiratory symptoms progressively improvement. Replacement therapy with PEG-ADA was started immediately at a dose of 30 U/kg/twice per week. Ig therapy was started at a dose of 200 mg/kg every two weeks. After three months of treatment patient showed an increase in T cells count (CD3, 411/mmc), and a decrease of toxic metabolites: AXP, 1.652 micromol/ml RBC; dAXP, 0.011 micromol/ml RBC; %dAXP, 0.7 maternal T-cell engraftment persists, despite a good response to the PEG-ADA therapy. The last examination before HSCT reveals maternal T-cell engraftment of 9.2%. Patient underwent HSCT from MUD HLA-identical donor after a myelo-ablative reduced intensity conditioning regimen protocol D EBMT/ESID guidelines. The number of infused CD34+ cells was  $14.29\times 10^6$ /Kg and  $69.47\times 10^6$  CD3/Kg. She is actually at day+108 post HSCT, is doing well and shows 100% engraftment of donor cells.

**Disclosure of conflict of interest:** None.

#### P479

##### Microbiome dynamics during stem cell transplantation in children using total gut decontamination as graft-versus-host prophylaxis

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Graft versus host disease (GvHD) is a frequent complication in patients undergoing haematopoietic stem cell transplantation. While the exact pathophysiology of GvHD is not known, the gut microbiome has been implicated in its development since it was shown that total gut decontamination (TGD) decreases the incidence of GvHD. With this study we aim to get insight into the diversity of the gut microbiota before, during and after total gut decontamination in comparison with selective gut decontamination (SGD). Secondly, we want to identify changes in microbiota composition that relate to the occurrence of graft-versus-host disease. For this prospective cohort study we recruited 22 children (<18y) that were eligible for a stem cell transplantation at the Leiden University Medical Center between January and December 2015. Of these, 64% ( $n=14$ ) received TGD (consisting of piperacillin/tazobactam and oral amphotericin B), whereas the other 36% ( $n=8$ ) received selective gut decontamination with polymyxin/neomycin and oral amphotericin B. In total, 129 fecal samples were collected, weekly during admission for the stem cell transplantation and monthly thereafter up to 6 months after transplantation. Also samples were collected from family stem cell donors as healthy controls. Samples were processed within 24 hours and stored in the -80 freezer, after which 16s V4 amplicon sequencing (Illumina Hiseq, rapid mode, 250 bp read length) was applied. Data analysis (taxonomy and Shannon diversity) was primarily done using QIIME (ref). Compared to microbiota diversity in stem cell donors (mean Shannon index (SI) 3.43), we observed an overall lower mean SI during TGD (1.90) and slightly higher mean SI during SGD (2.43). Microbiota diversity months after SGD (2.45) was similar to diversity during SGD (2.43), while diversity months after TGD (2.63) was higher than during TGD (1.90). Further analysis of repopulation dynamics demonstrated no differences in repopulation duration after both decontamination strategies.

However, we did observe differences in the type of bacteria that repopulated, with *Bacteroidales* being more prominent in SGD and *Lactobacillales* more prominent in TGD patients. *Actinomycetales* (genus *Rothia*) was exclusively present in TGD patients during decontamination. Also, the *Clostridiales* (*Blautia*, *Lachnospiraceae* and *Peptostreptococcaceae*) were bacteria that appeared after the decontamination period. Four patients (18%) in this cohort developed GVHD grade 1 or more. In these patients we did observe individual compositional changes of the gut microbiota at the time of GVHD diagnosis, e.g. very low diversity or dominance of *Enterobacteriales*. Considerable microbiota diversity is observed in patients that received TGD. Different repopulation dynamics were observed between TGD and SGD. No common pattern was found in the GVHD cases.

**Disclosure of conflict of interest:** None.

#### P480

### Minimal residual disease (MRD) pre- and post- HCT for children with AML is highly predictive of event-free survival: a pediatric blood and marrow transplant consortium study

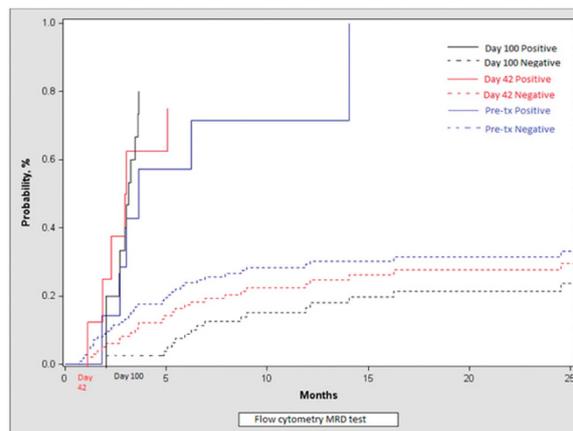
D Jacobsohn<sup>1</sup>, M Loken, M Fei<sup>2</sup>, A Adams, L Ejdenschen Brodersen<sup>3,4</sup>, W Fritschle<sup>4</sup>, B Logan<sup>5</sup>, K Woo Ahn<sup>5</sup>, B Shaw, M Kletzel<sup>6</sup>, M Olszewski<sup>7</sup>, S Kahn<sup>7</sup>, A Keating<sup>8</sup>, A Harris<sup>9</sup>, P Teira<sup>10</sup>, S Margossian<sup>11</sup>, R Duerst, P Martin<sup>12</sup>, M Boyer<sup>13</sup>, E Nemecek, C Dvorak<sup>14</sup>, A Petrovic<sup>15</sup> and M Pulsipher<sup>16</sup>

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Multicenter data regarding the significance of MRD in children with AML pre- and early post- HCT are lacking. We hypothesized that pre- and post-HCT MRD assessments using *WT1* PCR combined with multi-dimensional flow cytometry (MDF) would be predictive of disease relapse and event-free survival (EFS) at 2-yr post-HCT. Subjects were < 21 yrs with AML in morphologic CR undergoing MA allogeneic HCT. Stem cell sources included BM, PBSC, or CB. BM and PB samples were collected at 3 time points: baseline (< 3 weeks prior to preparative regimen); Day+42 (±14 days); and Day+100 (±20 days). BM samples were analyzed for both *WT1* expression and MDF MRD (single reference lab using a 'difference from normal' approach without access to diagnostic phenotype); PB samples were analyzed for *WT1* only. MDF detection limit was 0.02%; however, we required that 2 independent analysts certify that the abnormal population was AML. In addition, sorted MRD+ cells were tested for chimerism. *WT1* positivity was defined as ≥ 1300 copies for BM and ≥ 200 copies for PB. Results were not available to the treating clinician. 150 subjects were enrolled at 34 centers in US and Canada. 20 enrolled subjects did not undergo HCT and 6 were excluded for progression prior to HCT or other ineligibility. In 124 eligible subjects, 2-yr EFS and OS were 52% and 61%, respectively. The 2-yr CI of relapse and TRM were 36% and 13%, respectively. MDF identified 7 subjects pre-HCT having 0.2–14% residual disease. The 2-yr relapse rate in subjects with +MRD by MDF pre-HCT was 100% vs 32% (23–40%) in those who were negative. 2-yr DFS and OS were 0% and 29% (4–65%) for positive MDF pre-HCT, and 54% (45–63%) and 62% (53–71%) for negative MDF. Pre-HCT MDF

sensitivity for 2-yr DFS was 10%; specificity was 100%. MDF MRD at days 42 and 100 were similarly predictive of outcome. Sorted MRD+ cells from 19 post-HCT samples were all noted to be of recipient origin. PB *WT1* had no correlation with DFS or relapse; BM *WT1* at Day+100 correlated with 2-yr OS (79% (68–88%) low/neg vs 57% (39–75%) high). Other *WT1* cutoffs studied demonstrated no correlation with outcomes. Figure 1: Relapse probability by flow cytometry MRD at 3 time points. MDF MRD pre-HCT and at Days +42 and +100 was significantly associated with lower EFS and OS in children with AML undergoing HCT. MDF is specific but not sensitive, as many negative MDF patients relapsed. Our goal was to define a reproducible assay that did not depend on having the initial AML profile. This would facilitate multi-institutional studies aimed at decreasing relapse. Given that constraint, we were able to detect clear MDF MRD in a small percentage of patients that was highly predictive and can be used in trials. *WT1* level was not predictive in this multi-institutional trial. The sensitivity of flow was significantly affected by not having the initial flow available. Future attempts to improve sensitivity should include initial flow and/or test higher channel flow or molecular PCR techniques. In addition, we confirmed that MRD + cells obtained by cell sorting post-HCT were of recipient origin. Future testing of 'suspicious' sorted cells by FISH, molecular, or comparative genomic hybridization could possibly increase MFD sensitivity. Novel cellular or targeted therapies should be tested in clinical trials to improve outcomes in patients with MFD MRD noted either pre- or post-HCT.

[P480]



**Disclosure of conflict of interest:** None.

#### P481

### Novel mutations were identified with NGS and Low intensity of conditional regimen succeeded in children with Fanconi anemia who received allo-HSCT

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To explore the possibility of applying next-generation sequencing (NGS) to diagnose the disease of Fanconi anemia (FA) and evaluate the efficiency and safety of low intensity conditional regimen on children with FA receiving allogeneic hematopoietic stem cells transplantation (Allo-HSCT). Five patients initially suspected as severe aplastic anemia were diagnosed as FA by the method of next-generation sequencing (NGS)-based genetic diagnosis panel. One patient received HLA-identical sibling donor hematopoietic stem cell transplantation (MRD), three patients underwent unrelated donor matched (UD) HSCT, and one patient received unrelated cord blood transplantation (UCB). The conditional regimen consisted of

either 300 cGy TBI or 3.2–3.6 mg/kg of Busulfan with 20–40 mg/kg of cyclophosphamide. Meanwhile, ATG at 10 mg/kg and Fludarabine at 140–180 mg/m<sup>2</sup> were included as well. Cyclosporin or Tacrolimus as well as mycophenolate mofetil (MMF) were used for the prophylaxis of graft versus host disease (GVHD). Engraftment of neutrophil and platelet and complications followed transplantation such as infection, GVHD, and hemorrhagic cystitis (HC) were observed. Of 5 cases diagnosed as FA by NGS, only 1 case showed the abnormality of chromosome fragility test which has been regarded as golden criteria in the diagnosis of FA. Meanwhile, we found 5 novel mutations in 3 cases of FA which enriched Chinese national database with data of rare diseases by NGS. The counts of mononuclear cells (MNC) were (3.87–18.57) × 10<sup>8</sup>/kg for non-UCB and 9.83 × 10<sup>7</sup>/kg for UCB. The counts of CD34+ were (4.01–9.57) × 10<sup>6</sup>/kg for non-UCB and 2.56 × 10<sup>5</sup>/kg for UCB. All 5 cases succeeded in Allo-HSCT with the low intensity of conditional regimen. The median time for neutrophils engraftment was 11 days (range 9–15 days), median time to platelets (Plt) engraftment was 14 days (range 8–28 days). One case occurred with grade I of aGVHD, 2 cases with hemorrhagic cystitis. After transplantation, all patients were monitored the copies of EBV-DNA and CMV-DNA of whole blood, and five case with EBV positive and 3 cases with CMV positive. No patient suffered of EBV or CMV disease. The hepatic veno-occlusive disease (VOD) and HC were observed in 5 FA patients after transplantation. NGS showed much more specific and facilitated for the diagnosis of FA. Low intensity of conditional regimen is efficient and safe which should be recommended for the treatment of FA patients.

**Disclosure of conflict of interest:** None.

#### P482

##### **Outcome of alternate donor stem cell transplantation in children: An Indian experience**

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In India due to lack of donor registries and cord blood banks very few alternate donor stem cell transplants (SCT) are performed. Haploidentical SCT has become feasible with availability of post-transplant cyclophosphamide (PTCy) technique. Here we present our experience of setting up alternate donor program for SCT for children in India and report the outcomes of the same. We collected data retrospectively of all children who underwent alternate donor SCT during Jan 2013 to Dec 2016 in two centres. A total of 47 SCT were performed for 43 children; median age 6 years (1–18 years) and 37 were boys and 6 girls. Of these, 41 underwent haploidentical (35 PTCy and 6 TCR alpha-beta/CD19 depleted), 4 matched unrelated donors (MUD) and 2 unrelated cord blood (UCB) SCT. The diagnosis was: Primary Immunodeficiency-10, Thalassemia Major-14, Sickle Cell Disease-3, Inherited Bone Marrow Failure-4, Acquired Aplastic Anemia-3, Acute Lymphoblastic Leukemia-3, Acute Myeloid Leukemia-4, Neuroblastoma-4, Ewings Sarcoma-1 & Leukodystrophy-1. The conditioning was with Fludarabine, Cyclophosphamide and Total Body Irradiation backbone in 36 children (Thiotepa added in 24), Fludarabine and Treosulfan in 5, Fludarabine and Busulfan in 2, Busulfan and Cyclophosphamide in 4. Serotherapy was part of conditioning, Rabbit Anti-thymoglobulin 4.5 mg/kg in 38 and Campath 1 mg/kg in 9. Graft vs host disease (GVHD) prophylaxis was PTCy along with tacrolimus and mycophenolate mofetil in 37 patients (35 haploidentical, 1 MUD & 1 UCB) and ex-vivo TCR alpha-beta depletion in 6 and cyclosporine and methotrexate in 4. All were transplanted after a signed informed consent. A median of 8 million of CD34 cells/kg was infused (range 5–24 million/kg). Graft source was peripheral blood in 39 and bone marrow in 6 and UCB in 2. Five children died before engraftment. The remaining 42 had neutrophil engraftment by median of 14 days (range

8–35) and platelet engraftment by median of 14 days (range 9–48). Chimerism at day+100 was available in 34 cases; 31 of them had full donor hematopoiesis. One had mixed chimerism and 3 fully recipient. Four children underwent a second haploidentical SCT after rejection of which 2 are alive and disease free. The median follow-up of remaining patients is 9 months (range 1–40); the cumulative incidence of graft versus host disease (GVHD) acute and chronic extensive was 26% and 20% respectively. Grade-III–IV acute GVHD was seen in 3 patients. A total of 15 patients have died (sepsis-4, stroke-1, GVHD-3, VOD-3, encephalitis-3 and progressive disease-1). Among encephalitis deaths, one child had undergone UCB with PTCy and another TCR alpha-beta depleted second SCT; both had BK virus in the CSF. There were 11/41 deaths in Haploidentical (PTCy-10/35 & TCR alpha-beta-1/6), 3/4 in MUD and 1/2 in UCB SCT. Overall survival is 68% and disease free survival is 66% at last follow up. Alternate donor SCT is an acceptable curative option for children lacking a matched sibling donor. Haploidentical donor SCT is more feasible in the setting of lack of donor registries having Indian ethnicity donor.

**Disclosure of conflict of interest:** None.

#### P483

##### **Outcome of children with ALL in second complete remission transplanted from an unrelated donor: a retrospective analysis**

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Hematopoietic stem cell transplantation (HSCT) from an unrelated donor (UD) is largely used for pediatric patients with ALL in second complete remission (CR) lacking an HLA-identical sibling. In this study, we retrospectively analyzed outcome of patients (pts) given UD-HSCT in Centers affiliated to the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP) network between 2000 and 2013. Three hundred fifty-six pts with ALL in second CR experiencing either bone marrow (BM), isolated extramedullary or combined relapse were included in the study; 139 were males and 217 females, median age at HSCT being 4.8 years (range 0.5–19). BM, peripheral blood (PB) and cord blood (CB) were the stem cell source in 64%, 16% and 20% pts, respectively. All children received a myeloablative conditioning regimen, either TBI- (293 pts) or chemotherapy-based (63 pts). As GvHD prophylaxis, the combination of cyclosporine A, short-term MTX and ATG was employed in most pts. According to the Berlin-Frankfurt-Munster (BFM) classification of first leukemia recurrence, 59% and 42% of pts were assigned to the S1+S2 and S3+S4 groups, respectively. Level of pre-HSCT minimal residual disease (MRD), measured within 30 days before HSCT through flow cytometry (FCM) in the laboratory of Padova, is available in 37 children; more data will be presented during the

meeting. With a medium follow-up of 6.5 years (range 0.5–15), the overall survival (OS) was 52%, while the event-free survival (EFS) was 50%. The cumulative incidence of transplant-related mortality (TRM) and leukemia recurrence were 24% and 25%, respectively. The EFS probability for children transplanted in the time period 2000–2004, 2005–2009 and 2010–2013 was 45%, 56% and 52%, respectively ( $P=NS$ ). Patients who received a TBI-based conditioning regimen had a significantly better outcome in comparison to children who received chemotherapy-based treatment, EFS being 53% and 36%, respectively ( $P=0.01$ ). EFS of pts belonging to S1+S2 and S3+S4 groups was 66% and 35% respectively ( $P=0.0001$ ). The difference in EFS is largely explained by an increased incidence of leukemia recurrence in S3+S4 (34%) compared to S1+S2 pts (23%) ( $P=0.0002$ ). EFS of pts who experienced grade II acute GVHD was 68%, while that of pts with either absent/grade I acute GVHD or grade III-IV acute GVHD was 52% and 11%, respectively ( $P=0.0001$ ). Pts with limited chronic GVHD had a better EFS as compared to those with either extensive or absent chronic GVHD (77%, 35% and 61%, respectively;  $P=0.039$ ). The choice of stem cell source (BM, PBSC, CB) did not influence the probability of EFS, which was 56%, 46%, 40% respectively ( $P=NS$ ). Importantly, among pts with evaluable MRD before HSCT ( $N=37$ ), the group with detectable levels 0.001% ( $N=9$ ), respectively 54% and 17% ( $P=0.038$ ). Conclusions. Outcome of children with 2nd CR ALL who underwent transplant from an UD is significantly influenced by the presence of TBI in the conditioning regimen, limited severity of acute and chronic GVHD and BFM classification at time of 1st relapse. Notably, MRD level before transplant, namely with a cut-off of 0.001%, influences EFS.

**Disclosure of conflict of interest:** None.

#### P484

##### **Outcomes of autologous stem cell transplantation with CEAM conditioning in relapsed or refractory Hodgkin's lymphoma**

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Despite the generally excellent prognosis of children and adolescents with Hodgkin's lymphoma, approximately 20% of patients relapse. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a recognized treatment option for patients with relapsed Hodgkin's lymphoma. This study evaluates the results and outcome of non-cryopreserved autologous stem cell transplant of 25 patients with Hodgkin lymphoma. 30 patients age 4 to 25 years (median 13.5 years, M/F=19/11), with relapsed, refractory or poor prognosis HD, underwent ASCT in our hospital (from 2012 to 2016). Status at transplant was: second complete remission (CR2):  $n=15$ ; further CR (CR >2):  $n=12$ , partial remission (PR):  $n=3$ . All patients received chemotherapy-based conditioning regimens: cyclophosphamide, carmustine and etoposide (CBV): 6, CCNU(200 mg/m<sup>2</sup>) day -3, etoposide (800 mg/m<sup>2</sup>) day -3,-2, cytarabine(1000 mg/m<sup>2</sup>) day -3,-2 and melphalan(140 mg/m<sup>2</sup>) day -1 (CEAM): 24 pt's, Peripheral blood (PB) was the source of progenitor cells in 30 patients. All patients engrafted. The median mononuclear cell dose was  $5.5 \times 10^9$ /kg. The median time to reach absolute neutrophil count  $>0.5 \times 10^9$ /L was 13 days, and the median time to platelet count  $>20 \times 10^9$  was 16 days. Grade 2 and grade 3 mucositis was seen in 61% of our patients. Transplant-related mortality at 100 days not occurred. Only three patients relapsed 15, 18 and 30 months after transplant (mean 21.5 m.). With a median follow-up of 39 months (4–48 months) after transplant the event free survival were 84%. Only one patient had death, two years after transplantation. No significant difference between CBV group vs CEAM group in engraftment day. High-dose therapy with stem cell rescue can lead to durable remissions in children and adolescents with advanced

HD. Future investigations should focus on strategies designed to decrease relapse after auto-transplantation, particularly in patients at high risk for relapse. Our analysis suggests that these regimens (CEAM, CBV) are feasible in pediatric patients with acceptable engraftment and toxicity. PBSC collection may be difficult in small children owing to the large volume apheresis compared to the child's weight. Various problems, such as metabolic or haemodynamic disorders may be were seen. Peripheral Stem cell harvest can be performed in low-weight children under safe and effective conditions even when systematic priming by blood is avoided. Processing with increase of blood volume may to increase in the yield by recruiting progenitor cells.

**Disclosure of conflict of interest:** None.

#### P485

##### **Outcomes of children with hemophagocytic lymphohistiocytosis given allogeneic hematopoietic stem cell transplantation in Italy**

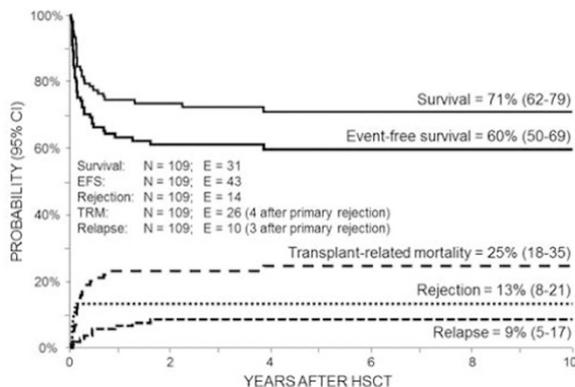
P Merli<sup>1</sup>, M Zecca<sup>2</sup>, F Fagioli<sup>3</sup>, A Rovelli, E Lanino, A Bertaina<sup>1</sup>, F Porta<sup>4</sup>, M Arico<sup>5</sup>, E Sieni<sup>5</sup>, G Basso, M Ripaldi<sup>6</sup>, C Favre<sup>5</sup>, M Pillon<sup>7</sup>, A Marzollo, M Rabusin<sup>8</sup>, S Cesaro<sup>9</sup>, M Caniglia<sup>10</sup>, P Di Bartolomeo<sup>11</sup>, O Ziino<sup>12</sup>, F Saglio, A Prete<sup>13</sup>, F Locatelli<sup>1,14</sup> and C Messina

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Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for patients with familial hemophagocytic lymphohistiocytosis (HLH) or relapsed/refractory HLH. We analyzed outcomes of a cohort of 109 patients (65 M, 44 F) with HLH given HSCT between 2000 and 2014. Median age at HSCT was 2 years (range 0.4–20). Genetic testing was performed for 94/109 patients (86%). Mutation of *PRF1* was found in 31 patients (32%), of *UNC13D* in 32 (33%), of *STXBP2* in 2 (2%), of *RAB27A* in 6 (6%), of *SH2D1A* in 5 (5%), of *BIRC4* in 2 (2%) and of *LYST* in 1 patient (1%). No known gene abnormality was found in 15 patients who had recurrent/refractory HLH. Central nervous system (CNS) involvement at diagnosis was recorded for 79 patients (72%) and was present in 30 of them (38%). The primary endpoint was event-free survival (EFS), defined as the probability of being alive and in continuous complete remission (CR) at last follow-up. In order to determine EFS, death from any cause, relapse or graft failure were considered events. Ninety-five patients received one transplant, while 14 received more than one HSCT, because of rejection in 8 patients or disease relapse in 6 (preceded by rejection in 1 case): 2 HSCT were performed in 12 cases, while 3 and 4 HSCT were performed in 1 case each. Donor for first transplant was an HLA-matched sibling for 25 patients (23%), an unrelated donor for 73 patients (67%) and a partially matched family donor for 11 patients (10%). Conditioning regimen was busulfan-based for 61 patients (56%), treosulfan-

based for 21 patients (20%) and fludarabine-based for 26 patients (24%). The 5-year probability of overall and EFS were 71% and 60% respectively (Fig. 1). Twenty-six (24%) patients died due to transplant-related causes, while 14 (13%) and 10 (9%) patients experienced graft rejection and/or relapse, respectively (see also Fig. 1). Twelve out of 14 children (86%) given a 2nd HSCT after graft failure/relapse are alive and disease-free. Active disease at HSCT was not statistically associated with adverse outcomes, while patients had a trend for a worse outcome if the interval between diagnosis and HSCT was >6 months. Patients transplanted from partially-matched family donors (PMFD) had a significantly worse EFS (9%) than recipients of a matched family donor transplant (73%) or a matched unrelated donor allograft (63%,  $P < 0.001$ ). The main reason for the dismal EFS of PMFD recipients was graft rejection, which, however, was largely rescued by a 2nd HSCT. Patients given peripheral blood stem cell transplantation had a lower EFS probability (39%) as compared to bone marrow (60%) or cord blood recipients (76%,  $P = 0.0185$ ). Children given HSCT < 6 months from diagnosis had a better EFS as compared to those transplanted >6 months from diagnosis (69% vs 50%,  $P = 0.069$ ). In multivariate analysis, only the use of a PMFD predicted a worse EFS probability (relative risk:12.26,  $P = 0.0008$ ). These data suggest that in patients with HLH allogeneic HSCT is able to cure 2/3 of patients. Haploidentical HSCT in patients with HLH is currently associated with unsatisfactory rate of engraftment, new approaches being needed to ameliorate this outcome. Active disease does not preclude the chance of benefiting from transplantation, which should be ideally performed within 6 months from diagnosis.

[P485]



Defibrotide shows efficacy in the prevention of sinusoidal obstruction syndrome (SOS) after allogeneic hematopoietic stem cell transplantation: a retrospective study on 237 patients. **Disclosure of conflict of interest:** None.

P486

**Post-transplant cyclophosphamide as graft-versus-host disease prophylaxis (GvHD) in pediatric patients with acute lymphoid leukemia (ALL) transplanted from haploidentical and matched unrelated donors**

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Standard GvHD prophylaxis regimens impair the graft-versus-tumor (GvT) effect, delay immune reconstitution and are associated with high rate of infections. High-dose post-transplantation cyclophosphamide (PTCy) targets alloreactive donor T cells proliferating early after BMT, promotes regulatory T cell and permits rapid immune reconstitution. In this pilot trial we evaluate the safety and effects of PTCy in unmanipulated haploidentical and matched unrelated transplantation (MUD) in pediatric patients with ALL. Fifteen pediatric patients with high risk ALL underwent unmanipulated allogeneic bone marrow (BM) ( $n = 11$ ) or peripheral blood stem cell (PBSC) ( $n = 4$ ) transplantation followed by PTCy between April 2014 and March 2016 with a median follow-up of 24 months (7–27). Eight patients were transplanted from haploidentical donors and 7 from MUD. The median age was 9.5 years (range 1.9–17) and were in complete remission (CR) at the moment of BMT. In 2 patients this was a second BMT. All pts. received myeloablative conditioning regimen (treosulfan-based  $n = 14$ , TBI based  $n = 1$ ) and PTCy on day +3, +4, posttransplant prophylaxis consisted of tacrolimus from day +5 ( $n = 5$ ), tacrolimus/MMF ( $n = 8$ ), ATG (rabbit, thymoglobuline) at 5 mg/kg without other posttransplant prophylaxis ( $n = 2$ , both from MUD). Primary engraftment was achieved in 100% of pts., the median time to neutrophil recovery was 20 days (16–45) and to platelet recovery was 22 (7–69) days. All pts. had full donor chimerism on day +30. Causes of death included viral infections ( $n = 1$ ); GvHD and viral infection ( $n = 1$ ). Cumulative incidence (CI) of acute GvHD grade  $\geq$  II was 33% (95% CI: 16–68), grade III–IV–13.3% (95% CI: 3.7–48) and chronic GvHD–21.2% (95% CI: 7.7–58.4). Two-year event-free survival (EFS) and overall survival (OS) were 86.7% (95% CI: 69.5–100) and were equal. Median time of follow-up for survivors is 2 years (range 0.7–2.3). We demonstrate that unmanipulated HSCT and posttransplantation cyclophosphamide allows for high rate of engraftment with acceptable transplant-related mortality in pediatric patients with ALL. All major outcomes were equivalent between transplantation from unrelated and haploidentical donor. GvHD prophylaxis including PTCy was effective. Event-free survival was high despite chemotherapy-based conditioning in most patients.

**Disclosure of conflict of interest:** None.

P487

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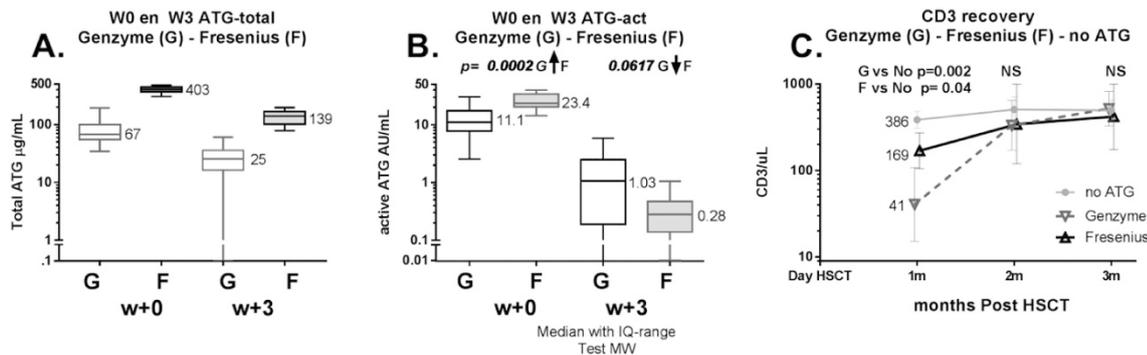
P488

**Rapid clearing of the active component of ATG-fresenius compared with ATG-genzyme impacts the immune recovery after allogeneic pediatric HSCT**

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Serotherapy with ATG is frequently used in allogeneic HSCT to prevent GvHD and rejection. However, the choice of the two most frequently used rabbit ATG brands depends on country, disease protocol, national recommendations and/or physician's preference. ATG-Genzyme (ATG-G, Thymoglobulin) is



prepared by immunizing rabbits with human thymocytes, whereas rabbits are immunized with a Jurkat cell line for production of ATG-Fresenius (ATG-F, recently named as anti-human T-lymphocyte immunoglobulin ATLG, Grafalon, Noveii Biotech). The recommended dose of both brands differs a factor 4–5. We have previously reported the pharmacokinetics/pharmacodynamics (PKPD) of ATG-G in a large cohort of pediatric HSCT recipients and concluded that the clearance of the active component of ATG, which is the portion of ATG binding to lymphocytes, had a major impact on immune recovery post-HSCT, while total ATG did not.<sup>1</sup> Both ATG brands have frequently been compared according to disease outcome, without detailed analysis of composition and clearance of the active components. In the present study, we compared clearance of the active component and immune recovery after ATG-G and ATG-F, respectively. The serum concentrations of total and active ATG were measured longitudinally after HSCT in 56 children (40 ATG-G, 16 ATG-F), transplanted with BM or PBSC of unrelated donors for ALL or AML between January 2010 and June 2016 in Leiden ( $n=36$ ) or Copenhagen ( $n=20$ ). ATG-G treated patients received a total dose of 6–10 mg/kg and ATG-F was given at a total dose of 30–60 mg/kg in both cohorts administration was divided over 3–4 days. Serum samples (pre-conditioning, day of HSCT, +1; +2; +3; +4 and +6 weeks and +2 and +3 months after HSCT) were analyzed by ELISA for total ATG and by quantitative flow cytometry on HUT78 cells for active (lymphocyte binding) ATG. Lymphocyte (sub-)populations were analyzed at +1, +2 and +3 months post-HSCT by flow cytometry. As reference group for immune recovery, 22 children transplanted for ALL or AML with an HLA-identical donor and not receiving serotherapy were included. The median serum concentration of total ATG at the day of HSCT was 6 times higher for ATG-F (ATG-G 67 µg/mL, ATG-F 403 µg/mL; Figure A) as the result of the higher dose of ATG-F given. The active ATG concentration was twice as high for ATG-F (ATG-G 11.1 AU/mL, ATG-F 23.4 AU/mL Figure B). Three weeks later at the expected time of engraftment, the total ATG concentration was decreased with the same factor for both ATG brands (ATG-G from 67 to 25 µg/mL, factor 2.7; ATG-F from 403 to 139 µg/mL, factor 2.9). However, the active ATG concentration showed a much faster decline for ATG-F (ATG-G from 11.1 to 1.03 IU/mL, factor 10.8; ATG-F from 23.4 to 0.23 IU/mL, factor 100). Correspondingly, the number of CD3 T-cells at 1 month post-HSCT was higher after ATG-F than after ATG-G (ATG-G, ATG-F and no-serotherapy 41, 169 and 386 cells/µL, respectively. Figure C). This is the first study to compare the PKPD of total and active ATG-Genzyme and ATG-Fresenius. Active ATG-F showed a much faster clearance than ATG-G, which was associated with a significantly faster CD3 T-cell recovery at 1 month post HSCT. Thus, ATG-F is not only quantitatively but also qualitatively very different from ATG-G, which will clearly impact HSCT outcomes.

**Reference**

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**Disclosure of conflict of interest:** None.

**P489**

**Reduced toxicity myeloablative conditioning regimen in pediatric hematologic malignancies not associated with improved outcomes**

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Allogeneic (Allo) hematopoietic cell transplantation (HCT) is the only curative potential therapy in refractory and relapsed pediatric leukemias. Poor outcomes in Allo HCT are associated with treatment-related mortality (TRM), mostly due to regimen-related toxicities (RRT) and graft-versus-host disease (GVHD) after myeloablative conditionings (MAC), but high relapse rate with reduced-intensity or nonmyeloablative regimens.<sup>1</sup> To improve TRM, without compromising conditioning intensity, we prospectively explored the feasibility and efficacy of a MAC but reduced-toxicity conditioning (RTC) regimen, consisting of fludarabine 30 mg/m<sup>2</sup>/d (given first) × 5d, daily busulfan dosed to target an AUC of 4000 microM\*min/d × 4, rATG 1.5 mg/kg/d × 3 and 400cGy of Total Body Irradiation in 30 patients (Table 1) with hematologic malignancies. GVHD prophylaxis was cyclosporine and MMF. All patients tolerated the RTC well, with no graft failures. RRT included moderate mucositis (67%), infections (bacterial 33%, viral reactivation 63%, fungal 20%) and 3 cases of venoocclusive disease (VOD). Cumulative incidence D100 ≥ Gr 3 acute GVHD was 32% (95% confidence interval [CI], 16–50), extensive chronic GVHD was 3.5% (95% CI, 0.2–16). Mortality at 100 days was 10.7% (95% CI 3–25), 2 due to infections with aGVHD and 1 VOD. With a median follow-up of 1.5 y (range, 0.6–5), the cumulative incidences of relapse at 1 years was 29% (95% CI, 14–47). Mortality due to severe aGVHD was 90%. Overall survival (OS) and progression-free survivals (PFS) for 1-year was 63% (95% CI, 42–78), and 56 % (95% CI, 36–72) respectively. On univariate analysis there was no association of outcomes with donor type, graft source, disease or busulfan exposure except significantly higher cGVHD in unrelated donors, aGVHD severity with peripheral blood. In summary, the use of the myeloablative RTC resulted in comparable TRM, with high relapse rate was high, including in those developing chronic GVHD. This suggested a less robust graft-versus-leukemia effect resulting in poor PFS and OS. Nonetheless, this regimen may be used as a lower-TRM platform to combine with other strategies, intensive disease monitoring pre and post HCT, addition of post HCT maintenance therapy in combination with marrow as the stem cell source to decrease relapse or GVHD.

[P489]

**Table 1 Patient and transplant characteristics**

Median Age at Transplant (yrs)	12.7(Range 2-20)
Gender	
M	13
F	17
Ethnicity	
Non Hispanic	16
Hispanic	14
Diagnosis	
Acute lymphoid leukemia (pre B)	8
Acute myeloid leukemia (AML)	16
Myelodysplastic Syndrome	3
Chronic Myeloid Leukemia	2
Biphenotypic leukemia	1
Remission Pre HCT	19
CR1( excluding MDS, CML)	5
CR2	1
CR3	
Median Time to Transplant (days)	180 d (55-492)
Donor	
HLA Matched Related Donor	11
HLA Matched Unrelated Donor	6
HLA Mismatched Unrelated Donor	5
Stem Cell Source	
Peripheral Blood Stem Cell	23
Bone Marrow	7
Median cell doses	
TNC dose (x10 <sup>8</sup> /kg)	4 ( 0.95-12.6)
CD34+ dose (x10 <sup>6</sup> /kg)	6.0 (2.45-13.0)
Median Engraftment (days)	
ANC > 500	13 (10-30)
Plt > 20K	16 (11-53)

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**Disclosure of conflict of interest:** None.

## P490

### Safety of bacille-carmette-guerin(BCG) vaccination after allogeneic hematopoietic stem cell transplan in children

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Specific immune response to vaccinations decline after hematopoietic stem cell transplantation (HSCT). Re-vaccination of all HSCT recipients is recommended in all guidelines but BCG vaccination is not recommended due to safety concerns after HSCT. Mycobacterium tuberculosis can cause severe disease in children including meningitis and millary tuberculosis (TB). The Bacille-Carmette-Guerin (BCG) is a live-attenuated vaccine with documented efficacy against millary disease and meningitis. Routine vaccination of all infants residing in countries with high TB incidence is recommended by World Health Organization. However, there is no data in

literature regarding its safety in post HSCT setting. Here, we report 34 children who underwent matched related allogeneic HSCT at Ankara University Pediatric Bone Marrow Transplant (BMT) Unit and received BCG 24-months post-transplant. All patients were free of graft versus host disease (GVHD) and immunosuppressive therapy (IST) and had negative PPD skin test prior to vaccination. None of the recipients developed local or disseminated tuberculosis as a complication of BCG with a median follow up of 10 years. We conclude that the BCG vaccine is safe in the post HSCT period when administered at least 24 months out of transplant to a selected group of patients who are free of GVHD and IST.

**Disclosure of conflict of interest:** None.

## P491

### Single centre experience of harvesting bone marrow from donors < 3 years of age

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Harvesting bone marrow for allogeneic marrow transplantation from donors < 15 kg presents special challenges. We present data on 67 sibling donors from our institution between 2006 and 2016. The mean age was 23 months with a range between 8 months to 48 months. Children less than one year accounted for 25% of our donors with the youngest being 8 months of age and the smallest donor weighed 5.5 kg. All aspirations were performed from iliac crests and all donors were given general anaesthesia by a paediatric anaesthetist. Irradiated blood was transfused in 97 % of the donors during the procedure. The volume of marrow obtained ranged from 5 to a maximum of 20 ml/kg donor weight. The product contained an average CD34 count of 5.5 × 10<sup>6</sup>/kg recipient weight with a range from 1.6 to 12 × 10<sup>6</sup>/kg. Only on one occasion was a second harvest needed, where the donor weighed 12 kg and recipient 42 kg with major blood group incompatibility requiring red cell reduction. The yield of CD34 cells per ml of bone marrow was on average 22% higher than children above 3 years of age. All recipients showed brisk engraftment in 2 weeks. None of these donors experienced major difficulties following the aspiration procedure. Thus, very young children may safely donate marrow for allogeneic transplantation and the yield of stem cells obtained is substantial. This data is particularly relevant in transplantation for haemoglobinopathies like thalassaemia major and sickle cell anaemia, where families are being counselled about a target of 15 kg for the donor in order to plan transplantation.

**Disclosure of conflict of interest:** None.

## P492

### Sinusoidal obstruction syndrome-veno-occlusive disease in pediatric patients given either autologous or allogeneic hematopoietic stem cell transplantation (HSCT). A retrospective study of the AIEOP-SCT (Italian haematology-oncology association-stem cell transplantation) group

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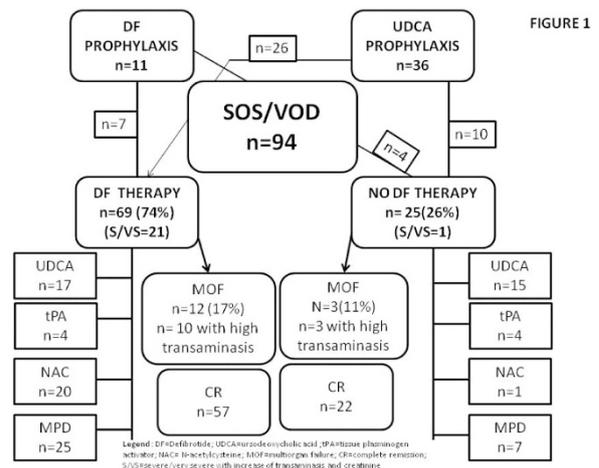
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Sinusoidal obstruction syndrome (SOS), known as veno-occlusive disease (VOD), is a potentially life threatening complication that can develop after HSCT. Although SOS progressively resolves within few weeks in most patients, the severe forms result associated with multi-organ dysfunction and high mortality rate (>80%). Aim of this survey is to evaluate incidence and management of SOS in a large cohort of children receiving either allogeneic or autologous HSCT. We retrospectively reviewed pediatric HSCTs performed in 12 (46%) out of 26 AIEOP affiliated centers, between January 2000 and April 2016. New EBMT criteria have been used for the diagnosis of SOS (serum total bilirubin  $\geq 2$  mg/dl and 2 of the following criteria: painful hepatomegaly, weight gain >5%, and ascites) and for the classification of severity grading.<sup>1,2</sup> Among a total number of 6208 HSCT procedures (2980 autologous and 3228 allogeneic), we identified 94 (1.5%) patients with SOS. This complication occurred in 37 and 55 cases after autologous and allogeneic HSCT, respectively. Fifty-two pts (55%) received iv Busulphan (BU) at myeloablative dose, 28 (30%) oral BU, while 14 (15%) were treated with different conditioning regimen. The median time of SOS occurrence was 16 days after HSCT. Details about prophylaxis and therapy are reported in Figure 1. Out of the 92 children, 54 (59%) fulfilled all SOS-EBMT criteria. Bilirubin  $\geq 2$  mg/dL, gain of weight >5%, ascites, and painful hepatomegaly did not occur in 16, 3, 4 and 2 patients, respectively. Thrombocytopenia was present in 85 pts (92%), thickening of gallbladder in 63 (68%) and abnormalities of coagulation parameters in 78 (84%). According to SOS EBMT severity grading, levels of transaminases were mild in 18 pts (19%), moderate in 21 (22%), severe in 13 (14.1%), and very severe in 42 (45.6%). Notably, creatinine was mild in 67 pts (71%), while 5 (5.4%), 10 (10.8%), and 12 (13%) children showed moderate, severe and very severe grade of renal failure. Thirty-three pts (36%) had respiratory failure, and 28 (85%) of them experienced right pleural effusion. Six out of the 25 patients who developed acute kidney injury, required dialysis. Severe encephalopathy occurred in 8 pts (8.6%) and 22 (24%) out of the 92 pts evaluated, were admitted in intensive care unit. As therapy of SOS, 69 pts received Defibrotide (DF); the dosage was 25 mg/Kg/day in 63% of them. The median duration of DF treatment was 15.5 days (range 4–104). Thirty-three (35%) pts received methylprednisolone (median dose of 2 mg/Kg). Fifteen pts (16.3%) died due to MOF (2 in moderate, 6 in severe, and 7 in very severe group) at a median time of 6 days from SOS diagnosis (range 3–75 gg). Our multicenter survey showed that, at least in our experience, there is a significant variability in the management approaches to SOS/VOD in children, while, diagnostic evaluations are more homogeneous. Interestingly, in our cohort, the increase of bilirubin may be an absent criteria, while thrombocytopenia and abnormalities of coagulation parameters are more frequent. As expected, MOF occurred mostly in patients experiencing severe SOS. DF represents first strategy to treat SOS in the majority of patients, even if steroids and ursodeoxycholic acid are still used.

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[P492]



Disclosure of conflict of interest: None.

## P493

### Successful allogeneic hematopoietic stem cell transplantation from unrelated donor for DOCK8 deficiency

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The hyper-IgE syndromes are characterized by marked elevations in plasma IgE levels and eosinophilia with impairment in T cells which clinically results in combined immune deficiency. DOCK8 deficiency, the autosomal recessive form, brings about allergic/atopic manifestations and unusual susceptibility to infections with herpesvirus family members (herpes simplex virus, human papilloma virus) and molluscum contagiosum. Symptoms in patients with DOCK8 deficiency typically emerge during childhood, and the majority results in death because of infections and malignancy by the third decade. Hematopoietic stem cell transplantation (HSCT) is now considered a standard of care for DOCK8 deficiency when an appropriate donor is available. In this study, we present the 5 unrelated HSCT results of 4 children with DOCK8 mutation. The demographic and clinical data of the 4 patients with 5 transplantations studied are shown in Table 1. HSCT was administered between August 2013 and August 2015 at Bahçesehir University Medical Park Antalya Hospital and the clinical data of the HSCTs are presented in Table 2. All patients were transplanted from unrelated donors with bone marrow, except one with cord blood. The cord blood transplantation's regimen was non-myeloablative which resulted with rejection. Despite existence of serious morbid problems before transplantation, all the patients engrafted successfully. Majority of the complications mentioned in the Table 2 were improved and they are in the follow-up in an outpatient basis. Discussion DOCK8 deficiency has high mortality, and HSCT should be considered as early as possible before development of significant organ damage. Despite myeloablative conditioning and high morbidity before the transplantation, survival was very good in our patients. Myeloablative and non-myeloablative transplants have been performed from related and unrelated donors and have reported successful results even without the preparative regimen. In our center, all

Table 1: The demographic and clinical data

Patient No	Gender	Clinical data	Transplant No	Age at transplant	Donor	Cell source
1	Female	Atopic dermatitis, pneumonia, otitis	1	4	MUD	BM
2	Female	Atopic dermatitis, pneumonia, otitis	1	11	MUD	BM
3	Female	Atopic dermatitis, pneumonia, skin infections	1	12	MUD	CB
4	Female	Same as 3, no deterioration after 1 <sup>st</sup> transplant	2	13	MUD	BM
5	Female	Atopic dermatitis, pneumonia, otitis, autoimmune hemolytic anemia, UTI, herpetic lesions	1	11	MUD	BM

Table 2: The clinical data of the HSCTs

Patient No	Conditioning	GVHD prophylaxis	TNC <sup>a</sup>	CD34 <sup>b</sup>	Neutrophil engr <sup>d</sup>	Platelet engr <sup>d</sup>	Chimerism %	Post-transplant outcome (months after transplant)
1	Bu-Flu-ATG	CsA-Mtx	4.2	5.2	18	19	99	16; CMV viremia, Zona
2	Bu-Flu-ATG	CsA-Mtx	2.2	1	15	16	98	30; PRESS, CMV viremia, skin and intestinal aGVHD, psychological problems, BK Cystitis, Otitis externa, herpetic dermatitis
3	Bu <sup>c</sup> -Flu-ATG	CsA-Mp	4.4	2.3	31	37	0	12; Cholelithiasis
4	Bu-Flu-ATG	CsA-Mtx	5.2	3.4	30	34	99	28; PRESS, CMV viremia, skin and intestinal aGVHD, Zona, osteomyelitis, aspergillosis
5	Bu-Flu-ATG	CsA-Mtx	5	4.3	22	24	100	20; Pneumonia, CMV viremia, Otitis, Catheter related thrombosis, Genital lesions, Transient pancytopenia

a:10<sup>6</sup>/kg (10<sup>7</sup>/kg in cord blood); b:10<sup>6</sup>/kg (10<sup>5</sup>/kg in cord blood); c: Non-myeloablative; d: engraftment day

transplants performed from unrelated donors by myeloablative regimen have been successful but have resulted in transplant rejection with cord blood transplantation after non-myeloablative regimen. In all of our patients, stable full chimerism has been detected, however mixed chimerism have also been shown to be useful in several reports. Whether HSCT also cures the autoimmune complications and reduces the risk of cancers is as yet undetermined. However, a myeloablative conditioning regimen followed by allogeneic hematopoietic stem cell transplantation from unrelated donors in DOCK8 deficiency results in improvement of the clinical phenotype with a low incidence of regimen-related toxicity.

**Disclosure of conflict of interest:** None.

#### P494

##### Successful bone marrow transplantation after myeloablative conditioning in a child with IPEX syndrome

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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare disorder. Although most patients present in infancy with a clinical triad of intractable

diarrhea, insulin-dependent diabetes, and eczematous dermatitis, some patients present with severe food allergies and other autoimmune manifestations. The disease is caused by mutations in the forkhead box P3 (FOXP3) gene, a transcription factor that is essential for the development and function of regulatory T (Treg) cells. This cells plays an essential role in controlling immune responses and preventing autoimmunity. Patients usually die in the first years of life without treatment. The only effective cure is hematopoietic stem cell transplantation (HSCT). Here we report a patient with IPEX syndrome who underwent HSCT after myeloablative conditioning. 9 months of age boy with the history of diarrhea, insulin-dependent diabetes, eczematous dermatitis, pneumonia, Coombs positive hemolytic anemia, referred to our hospital for investigation of immunodeficiency. On admission physical examination showed eczematous skin rash, submandibular lymphadenopathy, hepatosplenomegaly. Before HSCT the patients treated with immunosuppressive agents including methylprednisolone, mycophenolate mofetil and monthly intravenous immunoglobulin. Complete blood count revealed anemia (Hb: 7.7 g/dL), and eosinophilia (1900/mm<sup>3</sup>). Serum immunoglobulins were: Ig G: 1550 mg/dL (463–1006), IgM: 172 mg/dL (46–159), IgA: 60.9 mg/dL (17–69), IgE :1538 IU/mL. Lymphocyte subset analysis showed CD3 64%, CD4 22%, CD8 40%, CD16+56 13%, CD19 19%. FOXP3 gene analysis showed c.748\_750delAAG mutation. At the age of 1 year, patient underwent HSCT from his HLA matched sibling. Myeloablative conditioning regimen including busulfan (20.4 mg/kg) and fludarabine (160 mg/m<sup>2</sup>) was given to the patient. Cyclosporine A and methotrexate (day +1, day +3, day +6) were used as graft versus host disease prophylaxis. Bone marrow was used as the stem cell source and the number of CD34+ cells was 4.5 × 10<sup>6</sup>/kg. Neutrophil and platelet engraftment were achieved on day +13 and +35

respectively. Acute and chronic GVHD were not observed, but patient developed veno-occlusive disease treated with defibrinolytic, sepsis treated with broad spectrum antibiotics. Chimerism analysis showed %98 donor profile at the third month of HSCT. After HSCT, autoimmune hemolytic anemia, eczematous dermatitis, food allergies, diarrhea and type 1 diabetes resolved completely within two months after HSCT. Now the patient is in good clinical condition without any symptoms 5 months after HSCT. Early HSCT provides better outcome in patients with IPEX, before the organ damage due to autoimmunity and/or adverse effects of immunosuppressive therapy. Myeloablative conditioning is associated with substantial transplantation-related mortality whereas nonmyeloablative conditioning carries an increased risk of rejection because of dysregulated effector T-cell function. In these patients, myeloablative conditioning was preferred because of the risk of rejection. Although the required levels of donor chimerism and conditioning intensity are unknown, engraftment of donor Treg cells seems to be sufficient to control the disease. The patient is well without any symptoms of IPEX after HSCT with full donor chimerism.

**Disclosure of conflict of interest:** None.

#### P495

##### **Successful haplo-identical stem cell transplant after $\alpha\beta$ TCR+ depletion in a patient with interferon gamma receptor 1 deficiency**

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Interferon gamma receptor 1 deficiency (IFNR1) is a rare autosomal recessive immune deficiency disorder associated with very poor outcome secondary to severe and disseminated mycobacterial infections. Hematopoietic stem cell transplantation (HSCT) has been proposed as a curative option. However, HSCT for these patients is particularly difficult owing to a high rate of graft rejection. The use of a non T-cell depleted transplant from an HLA-identical sibling and fully myeloablative conditioning regimen has been shown to have improved outcomes. We report the first successful HSCT with a T-depleted haplo-identical donor, performed in a girl with severe IFNR1 deficiency. We reviewed the medical chart of a 2-year-old Hispanic girl with IFNR1 deficiency who was diagnosed at birth, since her brother had previously been diagnosed with the same complete IFNR1 deficiency. They were found to have a novel mutation variant detected at c.201-1G>T. As expected with this disorder, she developed disseminated infection with Mycobacterium abscessus infection at 2 months of age and was subsequently found to have Mycobacterium abscessus osteomyelitis. She was treated with multiple antibiotics including: amikacin, linezolid, meropenem and clarithromycin while tigecycline was added a few weeks prior to admission for HSCT. She was continued on this therapy until day + 30 following which antimicrobials were gradually weaned off. She was enrolled on the BP-004 trial, a multicenter, prospective phase III trial (enrolling both malignant and non-malignant diseases) evaluating  $\alpha\beta$ TCR +/CD19+ depleted haplo-transplantation followed by administration of BPX-501 T cells containing the iC9 suicide gene, (ClinicalTrials.gov NCT02065869). Her conditioning regimen included busulfan (4 mg/kg/day for 4 days) and cyclophosphamide (50 mg/kg/day for 4 days). Fludarabine, TLI (900 cGy). GVH prophylaxis with ATG/Rituximab. The patient received a graft with: TNC- $9.98 \times 10^8$  cells/kg, CD34+ cells- $16 \times 10^6$  cells/kg, and  $\alpha\beta$ TCR+ T cell content of

$4.78 \times 10^4$  cells/kg. As per protocol, since the  $\alpha\beta$  TCR+ T cells in the product was below threshold of  $1 \times 10^5$  cells/kg, she did not receive any post-transplant immune suppression. Bone marrow recovery occurred at day +10 with ANC  $> 500/\text{mm}^3$  and platelet recovery at day +18. Full engraftment with 100% donor chimerism based on cytogenetic analysis was observed at day +28 after transplantation and has remained stable. She is currently 14 months post-transplant, and has done well without major complications and or signs of mycobacterial infection. There is limited data in patients receiving HSCT for IFNR1 deficiency with very poor outcomes either relating to graft failure, transplant complications and progressive mycobacterial infection. To our knowledge, this is the first patient with IFNR1 deficiency transplanted successfully with a haplo-identical donor and alive without any active mycobacterial infection. This report suggests that using a highly immunopotent graft depleted of only  $\alpha\beta$ TCR+ T cells while retaining other immune effectors might offer a potential strategy to engraft these high risk patients using haplo-identical donors thereby allowing access to virtually all patients in need.

**Disclosure of conflict of interest:** None.

#### P496

##### **Tandem autologous stem cell transplantations for high risk pediatric embryonal central nervous system tumors: a single center experience**

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Pediatric embryonal Central Nervous System tumors are highly malignant tumors, which tend to disseminate through the cerebrospinal fluid to the brain and spinal cord and include: Medulloblastoma, Pinealoblastoma and Primitive Neuroectodermal Tumors (PNETs). The recommended treatment for these tumors is a complete surgical excision, craniospinal radiation and chemotherapy. The use of high dose chemotherapy with tandem autologous hematopoietic stem cell transplantation (HSCT) has been advocated for high risk patients, and infants who could not be irradiated. Between July 2010 and November 2016, 16 pediatric patients (11 males, 5 females) suffering from high risk medulloblastoma, PNET or pinealoblastoma underwent tandem autologous HSCT. They were treated according to two protocols: Group A consisted of ten patients with median age of 8.2 years (range 3.9–15.5 years) received the St Jude SJMB03 protocol, while group B consisted of six patients with median age of 2.1 years (range 1.4–3.5 years) who received the children's oncology group - ACNS0334 protocol. All patients engrafted with median time for neutrophil engraftment of 11 days (range 7–14 days) and for platelets engraftment ( $> 20,000$ ) of 13 days (range 13–24 days). Median follow-up was 3.5 years (range 1 week–6 years). Neurological toxicity: Two Group A patients had convulsions episodes, one occurred during infusion of cryopreserved stem cells, and the other was a result of progressive disease during the last course of HSCT. Gastrointestinal toxicity: seven patients required total parenteral nutrition due to mucositis. Diarrhea occurred in seven patients, two of them were diagnosed with Rota virus and two with clostridium difficile. Infectious complications: All patients suffered from at least one episode of neutropenic fever which was treated with broad spectrum antibiotics. There were 7 documented bacteremia in 6 patients. (1 Klebsiella pneumoniae, 1 Proteus mirabilis, 3 Staphylococcus aureus, 1 Streptococcus viridans and 1 Staphylococcus epidermidis). Metabolic complications: four patients in group A developed reversible syndrome of inappropriate anti-diuretic hormone secretion (SIADH) during chemotherapy, and all group A patients developed hypomagnesemia. Four patients died, one due to progressive disease, one due to early relapse 3 months post treatment, one due to late relapse 5 years post treatment and one due to sepsis 4 months post treatment. Another patient relapsed 1.5 years

post treatment, underwent surgery and radiotherapy and is now 3 years post therapy. Late effects: four group A patients developed endocrinological sequelae at a median of 20 months (range 17–21 months) and require hormone replacement therapy. Tandem autologous HSCT is a feasible treatment for pediatric high risk embryonal tumors, with good engraftment and acceptable toxicities using SJMB03 and ACNS0334 protocols, with overall survival of 75%. Long follow-up is needed in order to diagnose and treat late effects.

**Disclosure of conflict of interest:** None.

**P497**

**The diagnostic role of liver stiffness measurement in predicting hepatic veno-occlusive disease (VOD) in pediatric hematopoietic stem cell transplantation (HSCT)**

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VOD is a potentially life-threatening complication associated with HSCT in which immediate therapeutic action is crucial for patients' outcome. Liver stiffness measurement (LSM) using Fibroscan represents a non-invasive method to detect the grade of liver fibrosis and portal hypertension as in case of VOD. To evaluate the predictive potential of LSM in pediatric patients (pts) at risk for developing VOD, a prospective, ongoing, single-center study has been performed at the University Hospital of Bologna. LSM was performed by using the Fibroscan device, which consists of a 3.5 MHz ultrasound transducer probe that transmits low-frequency vibrations (50 Hz) to the liver tissue. The propagation velocity is proportional to the stiffness (elasticity) of tissue. LSM will obtain pathological high values (> 7.5 kPa) when the tissue is altered like in liver fibrosis, or post-sinusoidal portal hypertension. From November 2014 – September 2016, 25 pediatric pts (18 male, 7 female), aged 3–20 years (mean 11.7), affected by hemato-oncologic disease, eligible to allogeneic (22) or autologous (3) SCT conditioned with busulfan-based

chemotherapy, were enrolled. Pts were scheduled for 4 study examinations with LSM: at T0 (baseline) before chemotherapy, T1 (day 7–10 after SCT), T2 (day 17–20) and T3 (day 27–30). The diagnosis of VOD was defined according to modified Seattle/Baltimore criteria. Twenty-five pts were enrolled in the protocol, 22 of which were evaluable for the study (Pts characteristics Table 1). 4 out of 22 pts (18%) developed VOD. The cumulative incidence (SE) of VOD in our setting was 19% (8.6). Baseline LSM values on T0 of all pts were normal (7.5 kPa at T2 ( $P=0.002$ ) and T3 ( $P=0.004$ )). From our observations, an anticipating pattern of pathological LSM in presence of clinical and laboratory parameters within normal ranges in patients who develop VOD can be derived. Preliminary data indicate a high predictive potential of LSM in the diagnosis of VOD, however the number of cases is not sufficiently representative to draw definitive conclusions. To optimize the predictive potential of the method, more frequent (daily) measurement in the critical time frame are currently investigated.

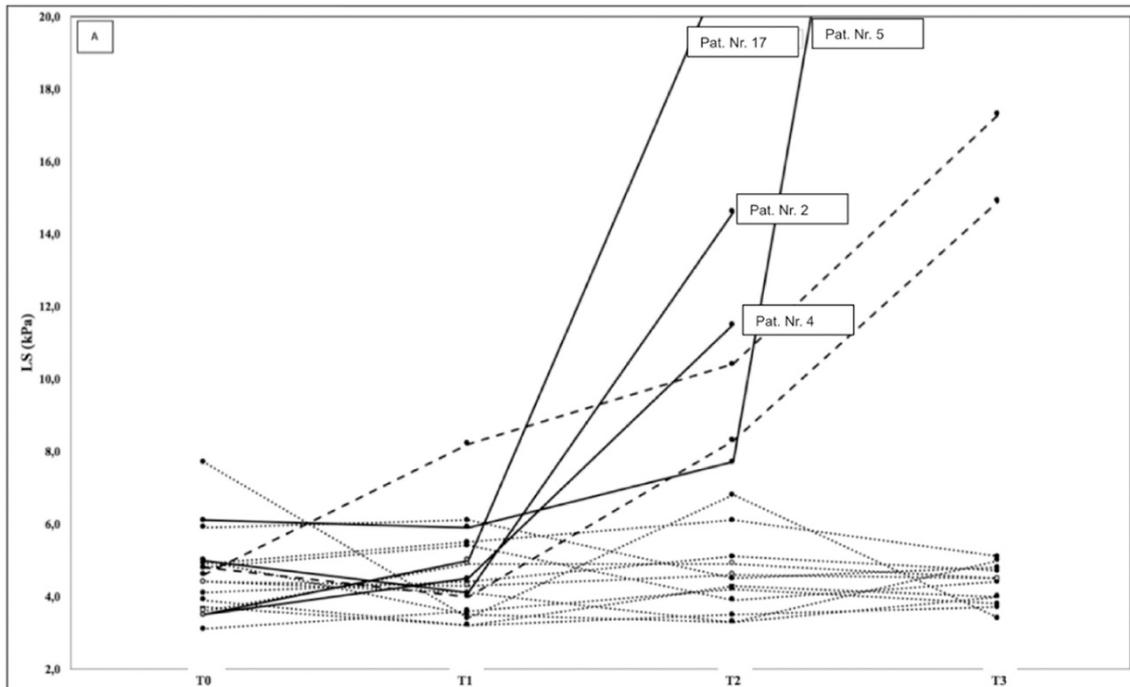
[P497]

**Table 1. Characteristics of evaluable patients**

	Frequency	(%)
<b>Diagnosis</b>		
ALL/AML	5 / 6	(22.8)/(27.3)
Ewing Sarcoma	3	(13.6)
Severe aplastic anemia	3	(13.6)
Beta-thalassemia	4	(18.2)
Hodgkin Lymphoma	1	(4.5)
<b>Conditioning regimen</b>		
BU-based	11	(50)
TREO-/FLUDA-based	8 / 3	(36.4)/(13.6)

ALL= acute lymphoblastic leukemia, AML= acute myeloid leukemia, BU= busulfan, TREO=treosulfan, FLUDA= fludarabine.  
**Disclosure of conflict of interest:** None.

[P497]



#### P498

### The exact role of extra-corporeal photopheresis in children with GvHD: an unanswered question

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ECP continues to be a controversial treatment, probably due to the mechanism of action not being identified, the varying photopheresis procedures and treatment schedules, and the difficulty of conducting trials on relatively rare diseases with involvement of clinically heterogeneous organs. ECP was performed in our pediatric transplant center to 8 patients mean age of 12 years (4–18) diagnosed to have ALL (3 pts), Thalassemia (2 pts), Aplastic Anemia (1), Blacfan Diamond (1), Refractory Hodgkin Disease (1) following our internal protocol for 152 ECP sessions. Five of the patients had MUD, 3 had HLA id sibling transplants. Chronic GvHD was diagnosed in 2 of the patients 6 had acute GvHD. Skin was involved in all the patients, liver in 6 of the patients, lung in 3, gut in 6 and mucous membranes in 7 patients. The ECP treatment consisted essentially of three steps: (1) collection of MNCs from the patient, (2) processing of MNC buffy coat, and (3) return of MNCs to the patient. Collection was performed using a cell separator (Haemonetics MCS plus), processing two blood volumes. Our protocol provides for a maximum final MNC volume to be collected at 150 mL, with a hematocrit (Hct) value below 5%. The maximum procedure time was set at 180 min. The MNCs collected were adjusted to a constant volume of 300 mL by the addition of saline and 3 mL of 8-MOP in aqueous solution, to always obtain a final concentration of the drug of 200 ng/mL. The diluted buffy coat was transferred into a special UV-A-permeable bag (PIT-KIT medtech solutions), and UV-A radiation at 2 J/cm<sup>2</sup> was performed (UVA-PIT irradiator). The photoactivated MNCs were returned to the patient within 30 minutes using a blood transfusion set. During ECP procedure, patients' vital signs were monitored. Anticoagulation consisted in acidcitrate-dextrose Formula A set at a variable ratio (1:14–1:20) according to the patient's characteristics (clinical conditions, body weight, coagulation values) and platelet (PLT) count. Prophylaxis of hypocalcemia consisted of the administration of calcium gluconate (5 mL diluted in 5–10 mL saline) every 30 to 45 minutes. All procedure related side effects were recorded. During the reinfusion and postreinfusion phases, the patients were monitored for fever, chills, headache, rash, erythema, urticaria, itching and edema. No serious complication was detected. All the patients had also steroids, 4 had concurrent mesenchymal stem cells. ECP was applied on 2 consecutive days every 2–4 weeks which is continued for approximately 6 months followed by a maintenance schedule tapered to an every 2- to 4-weeks. The mean session cycle was 19 (6–51) between February 2015 to November 2016. The most commonly involved organ was the skin which demonstrated a response rate of 75%, followed by liver (66%), lung (25%), gut (18%) and mucous membranes (68%) The concurrent immunosuppression could be reduced during ECP therapy, and no increase in opportunistic infections was detected. 1/8 patient died after a relapse, 7/8 are alive with chronic mild GvHD. However, despite our good response rates, our understanding of ECP remains limited. Patients who suffer from acute and chronic GVHD have limited treatment options. ECP remains an important therapeutic option. Future basic, translational, and clinical research studies will provide a better understanding of its mechanism of action and optimize its therapeutic potential.

**Disclosure of conflict of interest:** None.

#### P499

### Tolerability and responses to ex vivo IL2 activated NK cells from haploidentical parental donors in paediatric patients with refractory leukaemia/lymphoma

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Prognosis for patients with refractory leukaemia/lymphoma ineligible for transplants and those who relapse post-transplant is poor. In adult settings, adoptive transfers of ex vivo IL2 activated natural killer ('aNK') cells from NK alloreactive donors, especially for NK sensitive cancers, has been successful in bridging patients to curative transplants.(1) This approach has not been reported in paediatric patients. We report our experience in 8 consecutive patients, of median age 9 (range, 1–15) years, with refractory leukaemia/lymphoma (AML, 2; ALL, 2; mixed phenotype acute leukaemia, 2; lymphoma, 2) who received 9 treatments with 'aNK' from haploidentical parental donors on institutional protocol, between Aug 2012 and 2016. Parents/legal guardians/patients provided informed consents as per institutional guidelines for donors and patients procedures. Donor lymphocytes harvested at steady state were CD3 depleted followed by overnight culture in IL2 before being infused into patients lymphodepleted with fludarabine and cyclophosphamide. Additional Rituximab were given to 3 patients and another received TBI 2 Gy. Subcutaneous IL2 injections at doses 1–3 mU/m<sup>2</sup>/dose started on D-1 and were planned for 6 doses, as tolerated. NK alloreactive donors (KIR-ligand mismatch) and KIR B/X genotype were available to all except 2 patients. Two patients were treated for post-transplant relapse; 1 of whom also received 'aNK' pre-transplant; other 6 patients had failed best conventional therapy including CD19/CD3 bispecific T cell engager (Blinatumomab) in 1. Lymphodepletion was well tolerated. A median TNC and CD56+ dose of 9.8 (range, 2.9 to 38) × 10<sup>7</sup>/kg and 1.8 (range, 1.2–18) × 10<sup>7</sup>/kg, respectively were administered. Cytokine release syndrome (CRS) was observed in 8 of 9 treatments (6 grade 1, 1 grade 3, 1 grade 4). The patient with DOCK8 deficiency, disseminated EBV+ cerebral lymphoma had grade 4 CRS and robust tumour lysis syndrome but succumbed to neurotoxicity. Of the 9 treatments, there were 7 responses, including the 2 given post-transplant. Excluding the 2 treatments given post-transplant and 2 non-responders, median peak donor chimerism was 93% (range, 7–100%) occurring at a median of 12 (range, 7–22) days. Five patients (4 responders, 1 non-responder) proceeded to transplants at a median of 43 (range, 35–96) days after 'aNK.' Responders had longer survival time compared to non-responders (median 314 vs 88 days). Two responders (25%) achieved sustained minimal residual disease (MRD) remission after transplants and are alive 138 and 380 days from 'aNK.' Five eventually died of their primary leukaemia/lymphoma; 1 from CRS. Our preliminary experience in a small cohort of 8 paediatric patients with refractory leukaemia/lymphoma showed that adoptive transfers of ex vivo IL2 activated NK cells from haploidentical parental donors were tolerable; with responses seen in 75% of patients; and 25% achieving prolonged MRD remissions after transplants. Patients with cerebral diseases might be at increased risks of neurotoxicity with this approach; and care must be taken in patient selection and the design of the lymphodepletion therapy.

#### References

1. Miller JS *et al.* *Blood* 2005.

**Disclosure of conflict of interest:** None.

#### P500

### Unrelated cord blood transplants for primary immunodeficiency diseases (PID)—Results from a single paediatric center in SE Asia

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Alternative donor choices are limited in multi-racial, multi-ethnic societies with small families such as Singapore. Unrelated cord blood transplant provides a feasible alternative

to patients lacking adult stem cell donors in children with primary immunodeficiency diseases. Method: We describe our experience using unrelated cord blood transplant (UCBT) for 9 children with PID from August 2005 to November 2014. During this period we performed HSCT for 12 children with PID: 9 with unrelated cord blood (75%); 2 with MSD and 1 MUD. Out of 9 cases of UCBT there were 5 Severe Combined Immunodeficiency (SCID), 2 Chronic granulomatous disease (GCD), 1 HyperIgM Syndrome and 1 Wiskott Aldrich Syndrome (WAS). The median age of transplant was 13.7 months (range 1.3 to 83.3 months). All presented with multiple infections ranging from disseminated BCG infection to parainfluenza /RSV /rotavirus infection to pseudomonas sepsis, staphylococcal endocarditis to pulmonary aspergillosis for SCID. Hyper IgM presented with pneumocystis carini pneumonia while CGD conditions presented with perianal abscess and fungal pneumonia. The child with WAS had life threatening GIT bleeding and a hemorrhage tracheobronchial cast removed after a failed initial extubation for gastroscopy. Conditioning regimes consisted of reduced intensive (Fludarabine based) conditioning regime for SCID and myeloablative regime for the rest. The median TNC dose was  $12.7 \times 10(7)/\text{kg}$  (range 4.2 to 22.5) and median CD34+ cells dose was  $3.68 \times 10(5)/\text{kg}$  (range 1 to 8.9). Results: All engrafted well except for one graft failure in CGD. He refused 2nd transplant and died 1.5 years post transplant from fungal pneumonia. Median engraftment time for neutrophil was 21 days (range 13 to 33) and platelet was 30 days (range 18 to 65 days). Grade 1 skin AGVHD occurred in one patient while another patient died of AGVHD of liver and lungs. Chronic GVHD was found skin and liver in one patient. TRM was 11 % (due to AGVHD). Median follow up was 1255 days (ranged 327 to 4109). Overall 5 years survival was 78%. Post-transplant complication with life threatening pneumonia was not uncommon. One patient developed biopsy –proven idiopathic interstitial pneumonitis and required ECMO for one month. He received immunosuppressive drugs including methylprednisolone, infliximab, oral Imatinib (TK inhibitor), azithromycin and nebulised becotide. He was weaned off oxygen after 2–3 months. Conclusion: Our limited experience showed unrelated cord blood is good source of stem cell for transplant in PID in a multiracial population. One case of Graft failure was likely due too low cell dose CD34 +cells dose  $1 \times 10(5)/\text{kg}$ . The expertise in ICU has enabled us to support several patients who presented with infective pneumonia pre-transplant and post -transplant. With better technology like alpha/beta depletion haploidentical transplant may be a better option to achieve engraftment earlier so as to avoid stormy post- transplant infections seen in unrelated cord blood setting.

**Disclosure of conflict of interest:** None.

#### P501

##### **Unrelated donor peripheral blood stem cell transplantation for children with acquired severe aplastic anemia—should we really avoid this option?**

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Matched sibling donor bone marrow transplantation (MSD-BMT) remains the first therapeutical choice in patients with acquired severe aplastic anemia (SAA). If there is no MSD available, the second option is immunosuppressive therapy (IST). If there is no response to IST, matched unrelated donor hematopoietic stem cell transplantation (MUD-HSCT) remains the ultimate curative therapy. Bone Marrow (MB) is the preferred graft source for both MSD- and MUD-HSCT in SAA. In spite of these recommendations, literature from developing countries suggest that PBSCs are used more and more frequently without compromising the transplant results, as they seem to be preferred graft source for donors in many countries incl. Poland. Therefore we analyzed the efficacy of

MUD-HSCT in children with SAA transplanted in our centre. Clinical data of 44 SAA and PNH children and adolescents (27 boys and 17 girls), who underwent MUD-HSCT between October 2000 and July 2016 were retrospectively analyzed. The median age was 11.2 years (range 0.7–20 years) According to the graft source, the patients were divided into PBSC group (37 patients) and BM group (7 patients). Four patients required second MUD transplant due to graft rejection. Overall survival for all patients was 66 %. Estimated 5-year overall survival (OS) was not statistically different between PBSC group and BM group [(68% vs 54% )  $P=0.42$ ]. There was no significant difference in OS between group who had IST before transplant and the group, who had an upfront transplant as a first line of therapy [65% vs 62%,  $P=0.79$ ]. The time to neutrophil and platelet engraftment was statistically longer in BM group than in PBSC group [(ANC 16 vs 15 days, PLT 29 vs 16 days, respectively)  $P=0.017$ ]. The incidence of grade III–IV acute graft-versus-host disease (GVHD) in PBSC group was similar to that in BM group [37% (14/37) vs 29% (2/7)]. The incidence of chronic GVHD in PBSC group was similar to that in BMT group [8% (3/37) vs 14% (1/7)]. Other transplant-related complications like heart failure, central nervous bleeding, incidence of infections were comparable within the two regimens. There were 15 deaths in the whole group. The main reason of death were infectious complications or multiorgan failure (MOF) in severely pretransfused patients in this historical cohort of patients. Unrelated donor PBSC in children and adolescents with SAA seems to be not inferior to unrelated donor BMT. The incidence of chronic GvHD was surprisingly low in SAA recipients of MUD PBSC. Increased morbidity and mortality due to infections was due to individual poor clinical situation of patients before transplant (i.e. fungal infections, contamination with resistant bacteria, prolonged neutropenia).

**Disclosure of conflict of interest:** None.

#### P502

##### **Unrelated peripheral blood stem cell transplantation in four children with dyskeratosis congenita after a fludarabine-based reduced intensity conditioning regimen: A single-center experience**

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Dyskeratosis congenita (DC) is characterized by the clinical triad of reticular skin pigmentation, nail dystrophy, and oral leukoplakia. The majority of patients with DC develop bone marrow failure (BMF), which is the main cause of death in DC patients. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for BMF associated with DC. Transplant-related morbidity/mortality is common, especially after myeloablative conditioning regimens. HSCT has been introduced into the management of DC, which has had remarkable clinical results. We report our experience in 4 children with DC who underwent allogeneic transplantation at a single medical center. Patients received a fludarabine-based reduced intensity conditioning (RIC), and the graft source was unrelated peripheral blood stem cells. Median age at the time of HSCT was 5.5 years (range, 4–13 years). The numbers of infused mononuclear cells and CD34+ cells were  $15.62 \pm 3.04 \times 10^8/\text{kg}$  and  $5.80 \pm 3.37 \times 10^6/\text{kg}$ , respectively. The median time of neutrophil and platelet recovery were 13.5 days (range, 12–17 days) and 21.5 days (range, 19–26 days). Two patients experienced grade II–III acute graft-versus-host disease (GvHD), and chronic GvHD was only observed in one patient. All four patients remained alive and transfusion independent at the median follow-up of 18.5 months (range, 9–31 months). Correction of previously existing physical defects was observed in two patients. Unrelated peripheral blood HSCT can be a curative option for DC. RIC based on the type of disease is important to

achieve successful HSCT. A larger sample size and extended follow-up of this rare patient population are needed to determine whether the changes in therapy will improve long-term survival.

**Disclosure of conflict of interest:** None.

### P503

#### Updated outcome of allogeneic haematopoietic stem cell transplantation in autosomal recessive hyper-IgE syndrome due to dock 8 deficiency: Single center experience

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Autosomal recessive Hyper-IgE syndrome due to DOCK8 mutation is a combined primary immunodeficiency, characterized by severe eczema, recurrent infections, and susceptibility to autoimmunity, malignancy, and multiple allergies, in addition to unusual high serum IgE level. DOCK8 patients tend to have a progressive severe clinical course with mostly fatal outcome during second to third decade of life without hematopoietic stem cell transplantation (HSCT). In our center we have a large number of DOCK8 patients. During a period of 11 years (2006–2016), we transplanted 14 patients with documented DOCK-8 mutation confirmed by molecular genetics. One patient did not receive any conditioning because of poor clinical condition and he died from severe cutaneous and gut GVHD and another patient received CBT with Bu/Flu with zero engraftment. The rest of the patients received HSCT from HLA full matched donor with chemoablation with Bu/Cy for all with 100% lymphoid and myeloid engraftment (STR). Among those patients who received chemoablation, GVHD developed in 6 patients mostly grade I and II. In addition 3 patients died: one died of severe GVHD and the other two died of sepsis. For DOCK8 patients we highly recommend early HSCT if fully matched donor is available to prevent the high mortality associated with the disease.

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**Disclosure of conflict of interest:** None.

## Experimental stem cell transplantation

### P504

#### Previously published

### P505

#### Excellent outcomes of KIR-ligand mismatched cord blood transplantations in pediatric patients with refractory malignant disease

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Alloreactivity triggered by interactions between killer cell immunoglobulin-like receptors (KIR) and natural killer (NK) cells plays a role in graft-versus-tumor (GVT) effects after hematopoietic stem cell transplantations (SCT). In particular, KIR-ligand mismatching between the donor and recipient might promote NK cell alloreactivity after unrelated cord blood transplantations (UCBT) in adult patients with acute myeloid leukemia (AML). Recently, it has been suggested that allogeneic NK cells could be the effector cells that mediate GVT effects after mismatched allogeneic transplants for refractory childhood solid tumors. We evaluated the cases of 9 pediatric hematology and oncology patients [4 (44%) AML, 1 (12%) myelodysplastic syndrome [MDS], and 4 (44%) neuroblastoma [NBL] patients] who underwent KIR-CBT between 2010 and 2016 at our institution. Among the 4 AML cases, one involved refractory disease (induction failure), and the other three involved relapsed AML (one patient relapsed after the 1st SCT because of 5q-). All 4 NBL patients underwent KIR-CBT followed by auto-peripheral blood stem cell transplantation (PBSCT) because of stage 4 disease. The MDS patient underwent KIR-CBT because of refractory anemia with excess blasts. KIR mismatching was defined as incompatibility between the donor KIR and recipient KIR ligand, and only inhibitory KIR that interacted with human leukocyte antigen (HLA)-Bw4, -C1, or -C2 group ligands were considered. The median age of the patients was 4 (range 2–18) years. All 4 of the AML patients were in complete remission (CR) at the time of the HSCT (CR1 = one case, CR2 = 3 cases). The MDS patient was in a non-CR state, and all of the NBL patients were in their 1st CR at the time of the HSCT. The AML patients received total body irradiation (TBI)-based conditioning (12 Gy TBI and 120 mg/kg cyclophosphamide [CY]), and the MDS patient received busulfan (BU)-based conditioning (19.2 mg/kg BU and 120 mg/kg CY). The NBL patients received reduced-intensity conditioning regimens (125 mg/m<sup>2</sup> fludarabine, 140 mg/m<sup>2</sup> L-PAM, and 2 Gy TBI). The CB exhibited HLA 2–4 locus mismatches (DNA typing), including at least one inhibitory KIR gene mismatch. The prophylaxis for graft-versus-host disease (GVHD) consisted of tacrolimus and short-term methotrexate. Anti-thymocyte globulin (ATG) was not used as a GVHD prophylaxis in any case. After the median follow-up period of 25 months (range: 4–54 months), all 9 patients were alive, and none of them had relapsed after the KIR-CBT. Although grade II–IV GVHD was observed in 6 patients (67%), it was controlled with prednisolone. Chronic GVHD was not seen in any case. The present findings suggested that NK cell alloreactivity plays a role in preventing childhood myeloid leukemia and NBL relapse after KIR-CBT. Although our results are limited, this report provides novel data to support further investigations into the use of KIR-CBT for the treatment of pediatric refractory malignant disease.

**Disclosure of conflict of interest:** None.

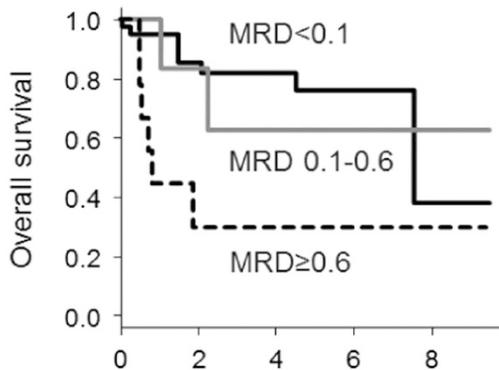
**P506**

**Impact of FCM-based minimal residual disease on transplant outcomes in patients with AML in hematological complete remission**

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It is reported that the presence of minimal residual disease (MRD) before hematopoietic stem cell transplantation (HSCT) is associated with poor overall survival in patients with acute myelogenous leukemia (AML) in hematological complete remission (CR). We retrospectively analyzed the association between flowcytometry (FCM)-based detection of MRD and transplant outcomes. We included 56 adult patients with AML in hematological CR, who underwent their first allogeneic HSCT between April 2005 and May 2015 at Kyoto University Hospital. MRD of bone marrow before HSCT was measured using FCM. To search for target antigens to detect MRD, three-color FCM analyses were performed using a differential panel for every disease and patient, which allowed us to detect  $\geq 0.1\%$  of MRD. Of the 56 patients (median age: 48.5, range: 18–66), 41 patients were included in the MRD-negative group (MRD  $< 0.1\%$ ), whereas 15 were included in the MRD-positive group (MRD  $\geq 0.1\%$ ). In the latter group, 6 patients were included in the MRD-low group (MRD  $< 0.6\%$ ), and 9 were included in the MRD-high group (MRD  $\geq 0.6\%$ ). There was no significant difference in the patient background between the MRD-negative and MRD-positive groups. The 3-year overall survival rates for the MRD-negative, MRD-low, and MRD-high groups were 82%, 63%, and 30%, respectively ( $P=0.007$ , Figure 1). In a multiple regression analysis, the MRD-high group was significantly associated with higher overall mortality than the MRD-negative group (MRD-low vs MRD-negative, hazard ratio [HR] 1.62,  $P=0.554$ ; MRD-high vs MRD-negative, HR 8.47,  $P < 0.001$ ). The 3-year relapse rates for the MRD-negative, MRD-low, and MRD-high groups were 15%, 0, and 67%, respectively ( $P < 0.001$ ). There were no significant differences in non-relapse mortality among the three groups. The analysis of FCM-based detection of MRD revealed that an MRD positivity of  $\geq 0.6\%$  was significantly associated with high risk of relapse and death even in patients with AML with hematological CR. The stronger consolidation or conditioning therapy before HSCT based on MRD could improve transplant outcomes in these patients.

[P506]



	Number at risk				
	0	2	4	6	8
MRD<0.1	41	24	14	9	1
MRD 0.1-0.6	6	4	3	1	1
MRD $\geq$ 0.6	9	2	2	2	1

**Disclosure of conflict of interest:** None.

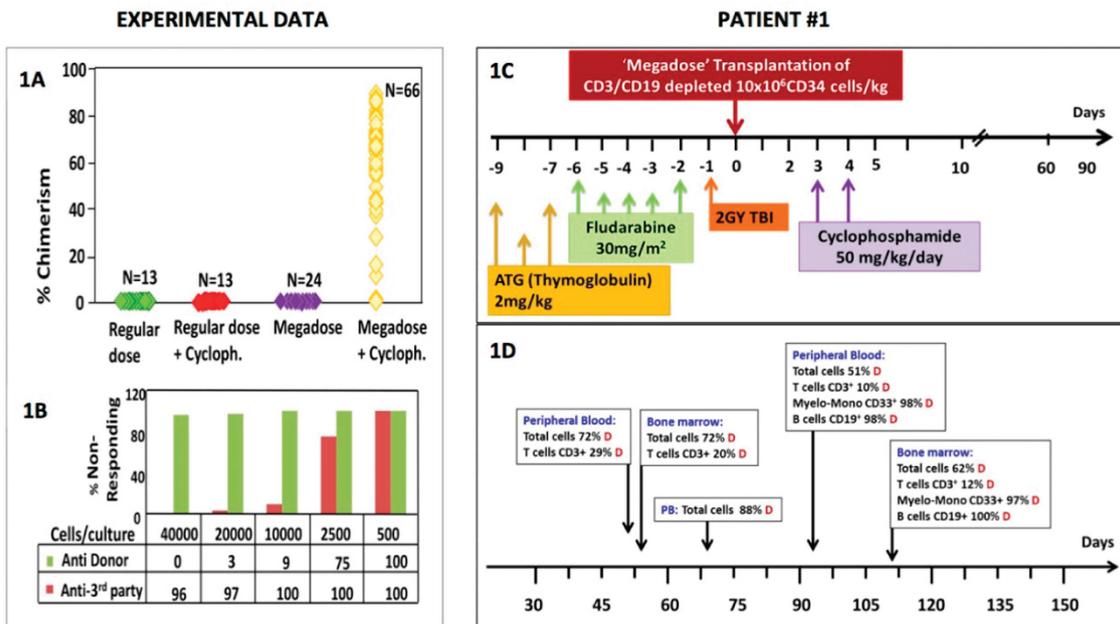
**P507**

**Post-transplant cyclophosphamide (PTCY) and megadose T cell depleted (TCD) haploHSCT for tolerance induction**

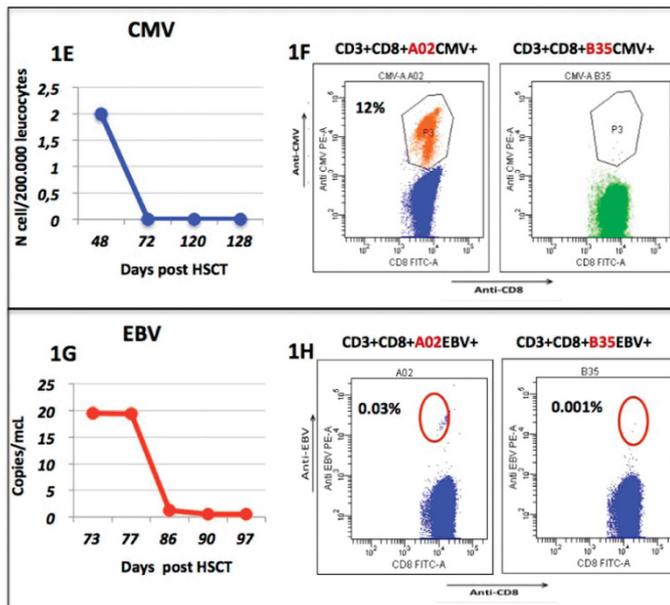
F Aversa<sup>1</sup>, E Bachar-Lustig<sup>2</sup>, N Or-Geva<sup>3</sup>, Y Zlotnikov Klionsky<sup>2</sup>, L Prezioso<sup>1</sup>, S Bonomini<sup>1</sup>, A Monti<sup>1</sup>, I Manfra<sup>1</sup>, C Schifano<sup>1</sup>, S Pratisoli<sup>4</sup>, F Lohr<sup>5</sup>, R Lamanna<sup>6</sup>, V Sgobba<sup>6</sup>, N Giuliani<sup>7</sup> and Y Reisner<sup>2</sup>

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The use of PTCY is associated with reduced risk for GVHD in T cell replete NMA haplo-HSCT; however, this intervention is still not sufficiently safe to justify treatment of non-malignant diseases or as a platform for organ transplantation. Experimental data: In a total of 66 mice, we showed that combining the power of megadose TCD HSCT with high dose PTCY (Fig.1A), enables marked and durable chimerism following NMA conditioning, while each modality alone was ineffective (Figure 1a). Chimerism included all myeloid and lymphoid lineages, and LDA analysis of alloreactive T cells revealed specific immune tolerance towards donor stimulators (Fig.1B), also associated with acceptance of donor but not 3<sup>rd</sup> party skin. Clinical trial: A similar protocol was developed for clinical use. The first patient, a 54 yr old male with high-risk multiple myeloma in CR after autoHSCT, received megadose ( $15.4 \times 10^6$  CD34+ cells/kg) CD3/CD19 depleted ( $1.17 \times 10^5$  CD3+T cells/kg) haploidentical PBPCs after ATG, Fludarabine and 2 Gy single fraction TBI. PTCY was given to control both HvG and GvH reactions (Fig. 1C). Hematopoietic engraftment was achieved at day +15 with over 97% donor type chimerism during the first 6 months in the myeloid and B cell lineages. T cells during this period were predominantly of host type (10–23% donor type), gradually increasing to 63–72% at 9–12 months post transplant (Fig. 1D). The patient overcame CMV and subsequently EBV reactivation without any treatment (Fig. 1E-1G). Dextramer FACS analysis revealed that CMV and EBV specific CD8 T cells were exclusively of host origin (Fig. 1F-1H). At +18 months, CR and normal Free Light Chain ratio were confirmed. The second patient, a 50 year-old male with high risk heavily pretreated multiple myeloma (tandem auto-HSCT, 3 yr maintenance with Lenalidomide, salvage therapy with VD) received a similar HSCT ( $10.8 \times 10^6$  CD34+ cells/kg,  $1.2 \times 10^5$  CD3+T cells/kg). Despite transient engraftment (50% donor cell on day +17), graft failure with autologous recovery (0.04% donor-type chimerism) was documented on day +30. This may be due to the extended treatment (3 yrs) with lenalidomide, but rejection cannot be excluded. After 5 months, this patient tolerated a second haplo-HSCT (different donor) after myeloablative conditioning (ATG, treosulfan, thiotepa and fludarabine) and alpha/beta TCR/CD19-depleted PBPCs. At 8 month follow up, he shows no sign of GvHD, good immunological reconstitution, excellent quality of life, and remains in complete remission. Collectively, our murine proof of concept data supported by clinical experience in the first high risk MM patient. The marked level of host T cells persisting over the first year after HSCT can provide anti-viral immune protection until thymus-derived donor T cells are generated. Avoiding additional post transplant immune suppression ensures a robust anti-viral immunity and a graft vs tumor effect. The rejection experienced by the 2<sup>nd</sup> patient, although corrected by a 2<sup>nd</sup> myeloablative TCD HSCT, indicates that the conditioning must be fine-tuned to optimize engraftment in every patient. We are therefore testing, increasing TBI from 2 Gy to 3 Gy. Further studies will determine the efficacy of this approach in elderly MM patients, in non-malignant hematopoietic diseases, or as a prelude for organ transplantation and cell therapy.



**Fig 1.**  
**A)** Chimerism induction in mice. Mixed chimerism was achieved only in recipients of the combined treatment.  
**B)** LDA of CTL-P performed against donor (green) or third-party (brown) splenocytes.  
**C)** Schematic representation of the haploidentical transplantation protocol combining megadose TCD HSCT and high dose PTCY;  
**D)** Chimerism analysis at different time points performed on peripheral blood and on bone marrow.  
**E,G)** CMV and EBV reactivation following HSCT and immune control of CMV (E) and EBV (G).  
**F,H)** Enumeration of Host origin (HLA A02) CMV (F) and EBV-specific (H) CD8<sup>+</sup> T cell with Dextramer kit, 55 and 110 days Post Transplant, respectively.



**Disclosure of conflict of interest:** None.

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## Acute leukaemia

### P508

**A comparative study on the effects of alemtuzumab in the matched unrelated donor (MUD) and sibling donor haemopoietic stem cell transplants (HSCT) settings, after fludarabine-based reduced intensity conditioning (RIC) regimes for high risk (HR) acute myeloblastic leukaemia (AML)**

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Over the last decade the addition of Alemtuzumab to fludarabine-based reduced intensity conditioning regimen is common practice in the unrelated donor allograft setting. In recent years, however, its use has extended to reduced intensity HLA-identical sibling donor allografts with the aim of providing an additional prophylaxis against GvHD. It is difficult to assess though whether this practice has any negative influence in the relapse rate or whether it has any net benefit or disadvantage in terms of overall survival. In this retrospective study we have analysed a historical cohort of 65 patients [41 males, 24 females, mean age 59.05 (40–73)] who

received a RIC fully matched unrelated donor (49 patients) or sibling donor (16) HSCT as consolidation treatment for HR AML in 2 transplant centres in UK and Greece. The conditioning regimen included fludarabine in all cases, together with melphalan and Alemtuzumab (29 patients), Busulphan and Campath (21 patients), busulphan and thiotepa (1 patient), Melphalan (3 patients), busulphan with and without ATG (9 patients) total body irradiation (200 cGy, 2 patients). In total, 50 patients received Alemtuzumab (35 MUD 50 mg Alemtuzumab and 15 sibling donor HSCT recipients 30 mg Alemtuzumab) and 9 patients (7 MUD and 2 sibling donor HSCT recipient) received ATG with 5 patients receiving T replete allografts. GvHD prophylaxis was Ciclosporin for patients receiving Alemtuzumab based or ATG based regimen and Ciclosporin with low dose methotrexate for t-replete allografts. The median follow up was 32.3 months (range 3–154 months). All but four patients were transplanted in CR1. Overall, patients receiving conditioning without Alemtuzumab suffered more frequent ( $P < 0.0001$ ) and more severe ( $P < 0.0001$ ) acute GvHD. This group, however, had a significantly ( $P < 0.05$ ) lower relapse rate. The overall survival remained unaffected. The subgroup of patients receiving allografts from MUD had a clear benefit in terms of a lower incidence ( $P < 0.0001$ ) and severity ( $P < 0.0001$ ) of acute GvHD: none of the patients receiving Alemtuzumab experienced grade IV aGvHD, but up to 5/14 patients not receiving Alemtuzumab suffered severe Grade IV GvHD. However, the use of campath was associated with a significantly higher rate of relapse or progression of the AML ( $P < 0.02$ ), so that none of the 6 MUD recipients not having campath relapsed, while 9/26 patients having Alemtuzumab relapsed. Although none of these factors had a net impact on survival, there was a non-significant ( $P = 0.07$ ) trend towards a higher survival in patients who received Alemtuzumab. In the sibling donor allograft setting, Alemtuzumab had no significant impact on the incidence of acute GvHD, relapse or survival. Finally, in diseases where cytogenetic or molecular markers of high risk were available, our results showed a better overall survival ( $P < 0.06$ ) in RIC Alemtuzumab conditioning undergoing fully matched unrelated donor HSCT, probably as a result of the protection against Graft versus Host Disease while maintaining Graft versus Leukaemia effect. Overall, Alemtuzumab is a highly protective agent against aGvHD in MUD HSCT recipients while it maintains the graft versus leukaemia effect. However it did not show any clear benefit of its use in the identical sibling donor setting. Larger prospective studies are required in order to determine the need for this agent in this particular setting.

**Disclosure of conflict of interest:** None.

#### P509

##### **A multicenter retrospective study of allogeneic hematopoietic cell transplantation in blastic plasmacytoid dendritic cell neoplasm**

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare disease which constitutes < 1% of all hematologic neoplasms annually. Majority of BPDCN present with diverse skin involvement prior to leukemic dissemination, whereas a minority (~10%) have systemic involvement at diagnosis. There are no established therapies for BPDCN and most pts receive acute leukemia, myeloid or lymphoblastic, induction regimens; but responses are short-lived and prognosis is poor upon relapse. Allogeneic hematopoietic cell transplantation (allo-HCT) is offered to BPDCN cases based on small retrospective or registry case series. We retrospectively analyzed outcomes of BPDCN pts who received an allo-HCT at 5 transplant centers in the USA. A total of 20 pts were eligible for analysis (Table 1). The primary endpoint was overall survival (OS). Twenty patients (M=18, 90%), median age of 51 (14–71) yrs, received an allo-HCT from a matched related ( $n = 9$ , 45%), matched unrelated ( $n = 7$ , 35%), mismatched-unrelated ( $n = 2$ , 10%), umbilical cord ( $n = 1$ , 5%) or haploidentical ( $n = 1$ , 5%) donor using myeloablative (MAC) ( $n = 13$ , 65%) or reduced-intensity (RIC) ( $n = 7$ , 35%) conditioning. Fifteen pts received hyper-CVAD as pre-allograft therapy (front-line = 14, salvage = 1). The majority ( $n = 17$ , 85%) were allografted in CR1. Median F/U for survivors was 26.3 (3.9–128.8) months. Median time-to-neutrophil and platelet engraftments were 16 (12–26) days and 15 (7–45) days, respectively. Five pts never dropped

#### [P509]

**Table 1.**

Variables	
Median (range) age of pts, yrs	51 (14-71)
Median (range) age, donors, yrs	25 (13-70)
Patient's gender	M=18 (90%) F=2 (10%)
Donor's gender	M=13 (65%) F=6 (30%) Cord blood=1 (5%)
Organ involved at diagnosis	BM=13 (65%) Skin=16 (80%) BM and skin=9 (45%) CNS=1 (5%)
Disease status at allo-HCT	CR1=17 (85%) CR2=3 (15%)
Cell source	BM=5 (25%) PBSC=14 (70%) Cord blood=1 (5%)
Donor source	MRD=9 (45%) MUD=7 (35%) MMUD=2 (10%) Haplo=1 (5%) Cord blood=1 (5%)
Regimen intensity	Myeloablative=13 (65%) Reduced-intensity=7 (35%)
GVHD prophylaxis	Methotrexate-based=7 (35%)* Mycophenolate mofetil-based=7 (35%)** Sirolimus-based=6 (30%)
CD34 cell dose, median (range)	4.6 (1.14-10.6)
HCT-comorbidity index at allo-HCT	0=7 (35%) 1=2 (10%) 2=7 (35%) ≥3=4 (20%)
Recipient/donor CMV serology	+/+ =6 (30%) +/- =2 (10%) +/undetermined=2 (10%) -/- =9 (45%) -/+ =1 (5%)

platelet counts below 20 000/ $\mu$ L. Three pts (MAC=2, RIC=1) relapsed at 6, 7, and 99 months, respectively. All 3 relapsed with marrow involvement (1 had also skin involved). Mean OS was 81.9 (53.1–110.8) months. One-year and 3-year OS were 85% (95% CI=64–95%) and 70% (95% CI=48–85%), respectively. There was no difference in 3-year OS when comparing MAC versus. RIC (HR=1.68 (95% CI=0.34, 8.4),  $P=0.53$ ). Median time to onset of acute GVHD was 35 (10–117) days; grade II–IV acute GVHD occurred in 6 cases. Chronic GVHD was seen in 5 cases (mild=2, mod/severe=3). Allo-HCT is an effective therapy for BPDCN resulting in durable remissions. Encouraging outcomes observed in this analysis may be explained by offering allo-HCT early in the disease course and in the setting of complete remission. Larger studies are needed to better understand risk factors for relapse to develop post-transplant strategies to improve outcomes.

**Disclosure of conflict of interest:** None.

## P510

### A risk-factor analysis for overall survival in patients with acute leukemia that relapse following T-replete haploidentical transplantation: On behalf of the acute leukemia working party of the european society for blood and marrow transplantation

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Relapse of acute leukemia is the leading cause of transplantation failure with devastating results. Relapse post T-replete haploidentical transplantations (haplo-SCT) is not well characterized. The objective of this study was to identify risk-factors for overall survival in patients with AL that relapsed after a haplo-SCT. From 2007 to 2014, 1660 haplo-SCT were performed in 186 EBMT centers as first allogeneic transplantations for adults with acute leukemia. Out of 657 patients for whom we were able to receive updated data, 208 relapsed and were included in this analysis. Median follow-up among survivors was 25 months after haplo-SCT (2–97) and 7.2 months (1–71) after relapse. Median time from haplo-SCT to relapse was 5 months (11 d–36m). Diagnosis was acute myeloid leukemia (AML) in 72% and acute lymphoblastic leukemia (ALL) in 28% of the patients, respectively. Fifty-two (25%) patients were transplanted in first complete remission (CR1), 42 (20%) in CR2 or CR3, while 114 (55%) were transplanted in active disease. RIC regimen was used in 116 (57%) patients and 85 (41%) received bone marrow as stem cell source. Post-transplant cyclophosphamide (PT-Cy) was used for Graft-versus-host disease (GvHD) prophylaxis in 121 patients (58%). Fifty-two (25%) of the patients who relapsed post haplo-SCT experienced previously acute GvHD and 42 (21%) chronic GvHD post transplantation. Treatment of relapse varied and included: none in 42 (21%), IST withdrawal only in 15 (8%), chemotherapy (CT) only in 59 (30%), tyrosine-kinase inhibitor (TKI) only in 2 (2%), TKI and CT in 6 (3%), DLI only in 9 (4%), subsequent transplant in 12 (6%), CT and DLI in 37 (19%), CT and subsequent transplant in 8 (4%), TKI CT and subsequent transplant in 1 (0.5%), DLI and subsequent transplant in 5 (2.5%) patients. Donors for second allogeneic transplant were unrelated ( $n=1$ ), haploidentical ( $n=23$ ) and cord blood ( $n=2$ ). Second transplant was performed in CR for 8 patients and in relapse for 18 patients. Only 2 patients who received a second haplo were alive at 47 and 69 months post second transplant. The majority of patients who received DLI were in relapse at time of DLI (81%), and 26% achieved CR after DLI. OS 1y after DLI was 27%, 7 patients being alive at a median time of 18 mo (4–55) post DLI. Overall, the one-year overall survival (OS) following relapse was 17% (95% CI: 11.6–22.3). In univariate analysis disease status at haplo-SCT (CR vs active disease), cytogenetics (good/intermediate vs poor) and median time from haplo-SCT to relapse ( $>$  or  $<$  5.03 months) were associated to a higher OS at one year after relapse: 27%

( $P=0.003$ ), 27% ( $P=0.03$ ) and 25% ( $P < 10^{-4}$ ), respectively. In multivariate analysis complete remission at haplo-SCT ( $P=0.004$ ; HR 0.64; CI:0.47–0.87) and time from haplo-SCT to relapse higher than 5.03 months ( $P=0.001$ ; HR 0.58; CI: 0.41–0.81) were risk factors for a higher OS after relapse. In the 58 patients transplanted in CR and relapsing more than 5 month after haplo, 1 and 2 y OS were respectively 33% and 20%. These findings suggest that similar to other transplantation setting OS for acute leukemia that relapse post haplo-SCT is dismal. Disease status at transplant and time from transplant to relapse are the two important prognostic factors that can predict somewhat better survival. Indication for second transplant should be carefully evaluated. Integrations with novel therapies are in unmet need to prevent and treat relapse post haplo-SCT.

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## P511

### Previously published

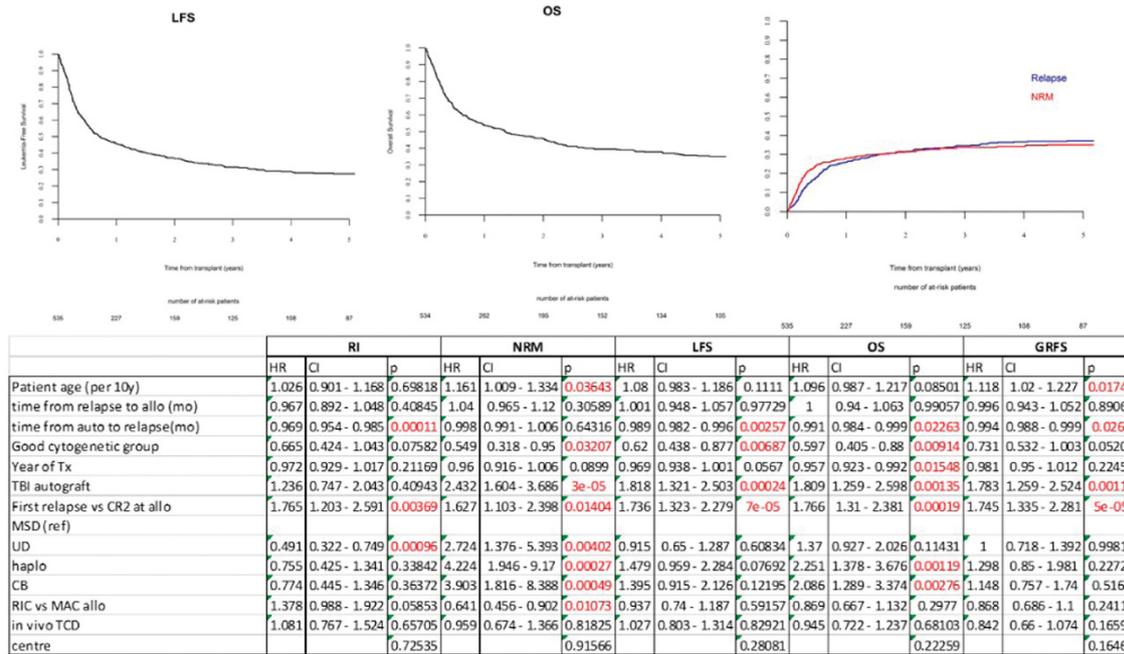
## P512

### Allogeneic stem cell transplantation following relapse post autologous stem cell transplantation in adult patients with acute myeloid leukemia (AML): a retrospective analysis of 537 patients from the acute leukemia working party of the EBMT

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Patients (pts) with AML who relapse after Autologous Stem Cell Transplantation (ASCT) have a dismal outcome but some can be rescued with an allogeneic transplantation (alloHSCT). Yet, available evidence presently stems from analyses of limited patient numbers. We decided to analyze the EBMT registry to evaluate the outcome and determine the prognostic factors in a large series of such pts. The EBMT registry was screened for adult pts with *de novo* AML (non-APL) who received an allograft in CR2 or first relapse (2000–2015) after being autografted in CR1. Pts receiving *ex vivo* T cell depletion (TCD) were included only if they received a haploidentical alloHSCT. Inclusion criteria were met by 537 pts (48% female, median age 45 [range 18–78] years). Median time from ASCT to relapse was 10 (range 0.6–176, IQR 5.8–19.1) months. At alloHSCT, pts were in 1<sup>st</sup> relapse (25%) or CR2 (75%). Donors were matched sibling (18%), unrelated (57%), haploidentical (13%), or cord blood (12%), respectively. Conditioning was myeloablative in 46% and reduced intensity in 54% of the pts,



respectively. The median follow up was 52 months (range < 1–167 months). At 3 years post allograft (figure), leukemia free survival (LFS) was 31.4% [95% CI 27.3–35.6], overall survival (OS) 39.5% [95% CI 35.1–43.9], relapse incidence (RI) 34.6% [95% CI 30.4–38.8], and non-relapse mortality (NRM) 33.7% [95% CI 29.6–37.9], while GRFS was 21.9% [95% CI 18.2–25.7]. Incidence of grade II-IV and III-IV acute graft vs host disease (aGVHD) was 26.3% [95% CI 22.4–30.2], and 12.5% [95% CI 9.8–15.7], respectively, while chronic (cGVHD) was observed in 30.6% [95% CI 26.4–35] and extensive cGVHD in 14.5% [95% CI 11.4–18] of the pts. All factors significantly associated with  $\geq 1$  endpoint in univariate analysis were entered in a multivariate Cox regression model (Table 1). RI was lower in pts transplanted in CR2 rather than in relapse (31.1% vs 44.7%; HR 1.76,  $P=0.004$ ) and in pts who relapsed later ( $> 10$  months, median value) as opposed to those who relapsed early post ASCT (25.5% vs 43.2%; HR (per month) 0.97,  $P < 10^{-3}$ ). RI was lower in pts transplanted with an unrelated donor (UD) in comparison to those transplanted from a matched sibling donor (29.1% vs 50.5%; MSD, HR: 0.49,  $P < 10^{-3}$ ). Patient age, poor cytogenetics, transplantation in relapse, previous TBI for ASCT, myeloablative conditioning (MAC) vs reduced intensity (RIC) and UD, haplo or CBT vs MSD all significantly increased NRM. LFS was significantly better in pts with good risk (47.3%) than in pts with intermediate risk or poor risk cytogenetics (29%; HR 0.62,  $P=0.007$ ) or in pts who relapsed late (per month: HR 0.99,  $P=0.003$ ) post ASCT. LFS was worse in pts who previously had received TBI (20% vs 45%; HR=1.82;  $P < 10^{-3}$ ). The same prognostic factors were significant for OS. Haploidentical (HR 2.25,  $P=10^{-3}$ ) and cord blood (HR 2.09,  $P=0.003$ ) transplants resulted in lower OS than those from MSD. Finally, date of transplant significantly influenced OS which was higher in pts transplanted after January 2008 vs those allografted before; 48.2% vs 31.7%, HR (per year) 0.96,  $P=0.02$ ). About one third of adult patients with AML who relapse post ASCT can be rescued with an allogeneic transplantation, especially if the duration of persisting CR post ASCT is long and no TBI was received in the past. Transplantation from an MSD while in CR2 rather than at relapse offers the best outcome.

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**P513**

**An IDA intensified conditioning haploidentical hematopoietic stem cell transplantation with combined GVHD prophylaxis of ATG and basiliximab improve survival for high-risk acute leukemia patients**

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High incidences of graft-versus-host disease (GVHD) and relapse have seriously impeded the widespread application of haploidentical hematopoietic stem cell transplantation (haplo-HSCT) for high-risk acute leukemia lacking conventional HLA-matched donors. One hundred and ten high-risk acute leukemia patients underwent haplo-HSCT with idarubicin (IDA) intensified conditioning regimen (IDA intensified BUCY2 for acute myelocytic leukemia (AML) and IDA intensified TBI-Cy for acute lymphoblastic leukemia (ALL)). For donor-recipient HLA 3/6 or 4/6 transplant, we separately administered a total of 9 mg/kg or 6 mg/kg antithymocyte globulin (ATG) and basiliximab for GVHD prophylaxis. All enrolled patients were observed longitudinally until death or lost to follow-up. The 100-day cumulative incidences of II-IV and III-IV aGVHD for all patients were 30.3%, 14.7%, respectively. The 2-year cumulative incidence of extensive cGVHD was 12.2%. The relapse rate was 18.2%. The 3-year probability of overall survival (OS) reached 63.1%. The patients in non-complete remission (NR) showed significantly higher relapse and worse survival than complete remission (CR) minimal residual disease (MRD) (-) and CR MRD (+) patients. However, the relapse, 3y-OS and disease-free survival (DFS) of CR MRD (-) did not differ from CR MRD (+) patients, indicating our intensified transplant technique could overcome the poor prognosis of MRD. For whatever AML or ALL patients, the relapse rates, aGVHD, cGVHD and the estimated 3-year OS and DFS between two ATG group were equivalent, except that ALL patients in ATG 9 mg/kg experienced higher relapse (33.0% vs 19.2%,  $P=0.226$ ). Although the incidence of cytomegalovirus (CMV) reactivation in ATG 9 mg/kg and 6 mg/kg was 77.4%, 72.9%, the average episodes of CMV reactivation were remarkably

higher in 9 mg/kg. Our IDA intensified haplo-HSCT technique could improve the outcome of high-risk acute leukemia and could be recommended as a good alternate for patients lacking HLA-matched sibling donors.

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#### P514

##### Analysis of post Allo-HCT relapse in acute leukaemia patients, a comparative on second Allo-HCT and donor lymphocyte infusions

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Acute Leukaemia relapse after Allogeneic Hematopoietic Cell Transplantation (Allo-HCT) associates poor prognosis. In this scenario, lowering the tumour burden prior to a Second Allo-HCT (2<sup>nd</sup> Allo-HCT) or Donor Lymphocyte Infusions (DLI) is essential to improve survival. Thus, patients that respond to chemotherapy and subsequently receive a DLI or 2<sup>nd</sup> Allo-HCT appear to associate better outcomes compared to patients receiving only chemotherapy, but data regarding this particular group of patients is lacking. We retrospectively analysed a cohort of post Allo-HCT relapsed acute leukaemia patients, who, after tumour reduction, were treated with either a 2<sup>nd</sup> Allo-HCT or DLI. Data was collected from 5 centers, 42 patients were consecutively included from 1995 to 2016. Patients were treated to reduce the tumour burden and received the 2<sup>nd</sup> Allo-HCT or DLI on morphological remission or post-chemotherapy aplasia. 26 patients (62%) were diagnosed with AML and 16 (38%) with ALL. 23 patients (55%) underwent 2<sup>nd</sup> Allo-HCT and 19 (45%) received DLI. Median patient age was 38 (4–66) years. The median follow-up was 674 (9–5823) days. Since data regarding time from first Allo-HCT to relapse was unavailable, we calculated the time from Allo-HCT to 2<sup>nd</sup> Allo-HCT or DLI (Time to 2<sup>nd</sup> Allo-HCT or DLI). Median Time to 2<sup>nd</sup> Allo-HCT/DLI was 336 (9–8823) days, and was 674 days and 336 days for 2<sup>nd</sup> Allo-HCT and DLI respectively ( $P=0.004$ ). Regarding the DLI group, the median DLI dose was  $1.1 \times 10^7 / CD3+$  ( $0.01-10 \times 10^7$ ) cells and the mean number of infused DLI was 1.4/patient. One-year OS was 51% (SE ± 8%). In OS univariate analysis, longer Time to 2<sup>nd</sup> Allo-HCT/DLI associated better survival rates ( $P=0.003$ ). The 1-year DFS was 39% (SE ± 8%). A longer Time to 2<sup>nd</sup> Allo-HCT/DLI ( $P=0.006$ ) and 2<sup>nd</sup> Allo-HCT compared to DLI ( $P=0.047$ ) (Figure 3) associated better DFS. The 1-year NRM was 24% (SE ± 8%). Univariate analysis identified PB as stem cell source as linked to better NRM ( $P=0.086$ ). The 1-year Relapse Incidence (RI) was 35% (SE ± 9%). RI univariate analysis related longer Time to 2<sup>nd</sup> Allo-

HCT/DLI ( $P=0.013$ ) to lower RI. On OS multivariate analysis, longer Time to 2<sup>nd</sup> Allo-HCT/DLI was associated to better survival ( $P=0.042$ ). This association was also observed on DFS multivariate analysis ( $P=0.017$ ). Table 1 summarizes 2<sup>nd</sup> Allo-HCT and DLI univariate analysis. Grade II-IV acute GVHD was diagnosed in 8 (35%) and 5 (26%) patients post 2<sup>nd</sup> Allo-HCT and DLI, respectively. Chronic GVHD was diagnosed in 8 (4 extensive) and 3 patients after a 2<sup>nd</sup> Allo-HCT and DLI, respectively. In this study, longer Time to 2<sup>nd</sup> Allo-HCT/DLI associated better DFS. 2<sup>nd</sup> Allo-HCT (compared to DLI) associated better DFS on univariate analysis, but this association was not observed on multivariate analysis. Of note, the 2<sup>nd</sup> Allo-HCT group included more patients with longer Time to 2<sup>nd</sup> Allo-HCT/DLI. This might be explained by 2<sup>nd</sup> Allo-HCT patients relapsing later or by the fact that the preparation of a 2<sup>nd</sup> Allo-HCT might require longer time than DLI. Results of this analysis warrant further study with larger number of patients.

[P514]

#### UNIVARIATE ANALYSIS

	2nd Allo-HCT±SE	DLI	Log-Rank p value
1-y OS	59% ±10	39% ±13	0.124
1-y DFS	50% ±11	24% ±18	0.047
1-y NRM	23% ±9	27% 14	0.973
1-y RI	24% ±11	49% ±15	0.140

**Disclosure of conflict of interest:** None.

#### P515

##### Analysis of survival after allogeneic transplantation by age strata for patients treated with CPX-351 liposome for injection versus cytarabine and daunorubicin 7+3 in patients aged 60–75 years with untreated high-risk acute myeloid leukemia

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Advancing age is associated with worse prognosis in acute myeloid leukemia (AML). Intensive induction chemotherapy in patients aged ≥ 60 years results in lower AML remission rates with increased induction mortality vs younger patients. CPX-351 is a liposomal formulation of cytarabine and daunorubicin encapsulated at a 5:1 molar ratio. A Phase III, randomized, open-label study of CPX-351 vs 7+3 (cytarabine and daunorubicin) in newly diagnosed older patients with high-risk secondary AML showed superior survival in the CPX-351 arm (hazard ratio 0.69;  $P=0.005$ ). In that trial, eligible patients went on to allogeneic hematopoietic cell transplantation (HCT). An exploratory analysis of those patients by age strata is reported here. Patients aged 60 to 75 years with newly

diagnosed secondary AML were randomized 1:1 to CPX-351 or standard 7+3 therapy. CPX-351 induction was 100 units/m<sup>2</sup> on days 1, 3, 5 (first induction) and days 1, 3 (reinduction); 7+3 first induction was cytarabine 100 mg/m<sup>2</sup>/day × 7 days and daunorubicin 60 mg/m<sup>2</sup> on days 1, 2, 3, and reinduction was cytarabine 100 mg/m<sup>2</sup>/day × 5 days and daunorubicin 60 mg/m<sup>2</sup> on days 1, 2. A dynamic allocation procedure stratified patients by age group (60–69 or 70–75 years) for each study arm. Patients with complete response (CR) or CR with incomplete platelet or neutrophil recovery were considered for allogeneic HCT, based on institutional criteria. Overall survival (OS) landmarked at the time of HCT was assessed. A total of 309 patients were enrolled on the induction trial. Arms were balanced for age with 153 patients (96 [63%] aged 60–69 and 57 [37%] aged 70–75) in the CPX-351 arm and 156 (102 [65%] aged 60–69 and 54 [35%] aged 70–75) in the 7+3 arm. In total, 91 patients received HCT: 52 (34%) in the CPX-351 arm and 39 (25%) in the 7+3 arm (odds ratio [OR] 1.54; *P* = 0.049). The HCT rate in the 60–69 group was 38% (36/96) in the CPX-351 arm and 32% (33/102) in the 7+3 arm (OR 1.25 [95% CI, 0.70–2.25]); in the 70–75 group, respective rates were 28% (16/57) and 11% (6/54) (OR 3.12 [95% CI, 1.12–8.75]). Patient and AML characteristics in the HCT age subgroups were generally similar between arms. In both age subgroups of patients receiving HCT, median OS was longer in the CPX-351 arm than in the 7+3 arm (Table 1). In the 60–69 group, serious adverse events (SAEs) prior to HCT in the CPX-351 and 7+3 arms occurred in 28% and 22% of patients, respectively; in the 70–75 group, in 13% and 67%, respectively. The most common SAE was febrile neutropenia (CPX-351, 11.5%; 7+3, 5.3%), occurring in all age groups.

[P515]

Table 1. Survival After HCT

	CPX-351 Median (95% CI)	7+3 Median (95% CI)	Hazard Ratio (95% CI)
Age 60-69	n=36	n=32*	
OS, months	NR (9.20-NR)	12.16 (5.78-24.25)	0.50 (0.25-1.01)
Age 70-75	n=16	n=6	
OS, months	NR (7.06-NR)	6.64 (0.85-NR)	0.22 (0.04-1.23)

\*1 patient not treated. NR, not reached. Overall and in both age groups, numerically more patients receiving CPX-351 underwent allogeneic HCT, and the transplant rate was significantly higher with CPX-351 in the ≥70 age group. In an exploratory analysis, OS after HCT appeared to be longer in the CPX-351 arm in both age groups. These results suggest that CPX-351 may provide an effective bridge to successful transplant for a high-risk subgroup of AML patients. Support: Celator Pharmaceuticals, Inc., a subsidiary of Jazz Pharmaceuticals plc.

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Pharma, Boehringer Ingelheim, Celator, Cyclacel, GlaxoSmithKline, Karyopharm Therapeutics, Sanofi, Sunesis Pharmaceuticals. Hogg: Consulting Sanofi; Travel Roche. Stone: Consulting AbbVie, Agios, Amgen, Celator, Celgene, Janssen, Juno Therapeutics, Karyopharm Therapeutics, Merck, Novartis, ONO, Pfizer, Roche, Seattle Genetics, Sunesis Pharmaceuticals, Xenetic Biosciences; Advisory Committee AbbVie, Celgene. Kolitz: Consulting Seattle Genetics, Pharmacyclics, and Gilead Sciences. Schiller: Research Funding Celator. Wieduwilt: Stock Reata Pharmaceuticals; Honoraria Alexion Pharmaceuticals; Research Funding Sigma-Tau. Ryan: Stock AbbVie; Patents University of Rochester. Chiarella and Louie: Employment Celator/Jazz; Stock Jazz Pharmaceuticals plc. Hoering, Newell, Lin, Solomon, Bixby: No relationships to disclose.

**P516**  
**Previously published**

**P517**  
**Azacitidine and donor lymphocyte infusion in relapsed AML or MDS after allogeneic stem cell transplant: a single-centre analysis**

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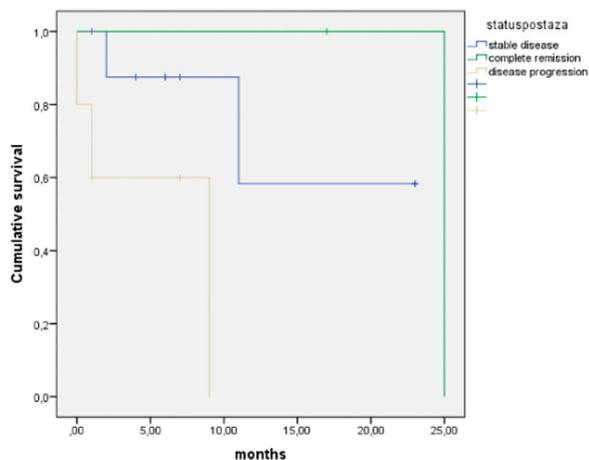
Relapse after allogeneic haematopoietic stem cell transplant (allo-HSCT) for acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) remains the main cause of treatment failure. It is associated with dismal prognosis and short survival. Proposed salvage strategies are tapering of immunosuppressive therapy, re-induction with chemotherapy and consolidation with donor lymphocyte infusion (DLI) or second allo-HSCT, although, results remain disappointing. Azacitidine (AZA) and DLI has proved to be an effective and well-tolerated outpatient approach in this setting, and results in at least temporary disease control in the majority of patients, thus, representing a valuable alternative to current treatments. Between January 2010 and November 2016, 16 patients with relapsed AML or MDS after allo-HSCT were treated with subcutaneous AZA 100 mg/m<sup>2</sup> days 1–5 every 28 days and escalating doses of DLI if feasible at Manchester Royal Infirmary, UK. AZA was continued until CR or disease progression. Patients characteristics: median age 60 (range 45–69) years, 56% males, diagnoses were AML (*n* = 12) and MDS (*n* = 4). Five (31%) patients had either monosomal or complex karyotype. Fifty percent of patients were in CR1 before transplant, 12.5% in CR2, 12.5% had a partial response and 25% did not receive any chemotherapy before the transplant. Fifteen out of 16 received Fludarabine-base reduced intensity conditioning regimen and all but one had a T-cell depleted graft. At relapse 88% had mixed donor chimerism. Median time to relapse was 9.5 (range 2–21) months after allo-HSCT. With a median follow up of 6.5 (range 1–25) months a median of 5 (range 1–16) courses of AZA were administered and median of 2 (range 1–6) DLI were infused. Doses of DLI were administered starting at 0.1 × 10<sup>7</sup>/kg and escalating by log5. AZA and DLI infusions were well tolerated; only two patients withdrew due to intolerance. Seven patients were admitted at least once due to infections (86%) or progressive disease. Only two patients developed mild GVHD grade 1. Complete remission was achieved in 12.5% patients and stable disease in 56%. Patients in CR had full donor chimerism. Median overall survival for patients in CR was 25 months compared to 9 months for those who did not respond (*P* = 0.031). Patients with more than 20% blasts on bone marrow at time of relapse after allo-HSCT had a worse outcome than those with less than 20% blasts (11 months and 25 months respectively, *P* = 0.09). No differences were seen when compared time to relapse (< 6 months vs ≥ 6 months)

and outcome, or disease and overall response, although numbers in this series are small. Image/Graph: Overall survival following azacitidine and DLI, patients in complete remission, stable disease and disease progression. Azacitidine and DLI can provide long term remissions in patients with relapsed AML/MDS post allo-HSCT with low toxicity. Lower disease burden at relapse carries better outcomes. Low rates of GvHD are seen following Azacitidine and DLI most likely showing the immunomodulatory effect of Azacitidine described by other groups.

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[P517]



**Disclosure of conflict of interest:** None.

#### P518

##### Case report: a patient with acute myeloid leukemia and dyskeratosis congenita undergoing allogeneic stem cell transplantation: Implications for treatment

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Acute myeloid leukemia (AML) is a frequent complication in patients affected by telomere maintenance disorders ('telomeropathies') such as dyskeratosis congenita (DKC). Treatment of AML in DKC patients by chemotherapy and hematopoietic stem cell transplantation is characterized by frequent remission failure, high organ toxicity and poor outcome. A 27-year-old patient with AML was admitted to our hospital in December 2014. He had been treated with 6 cycles BEACOPP for Hodgkin's lymphoma (HL) in 2009. On admission, the patient presented clinical signs of premature aging with hair greying and lack of fully recovered hair growth after chemotherapy (Cx) for HL. Flow-FISH analysis revealed TL below the 1% percentile within leucocytes in line with the suspected diagnosis of telomeropathy. Retrospective TL analysis by confocal Q-FISH from BM at HL diagnosis confirmed short TL before the start of any chemotherapy. He received standard AML induction Cx (3+7), but follow-up revealed persistence of AML. Salvage Cx with FLAG-Ida was applied resulting in partial remission with only weak

regeneration of normal hematopoiesis. The patient received an allogeneic stem cell transplantation (aSCT) after conditioning with 140 mg/m<sup>2</sup> melphalan and fludarabine from his HLA-matched brother whose TL was found to be normal. After aSCT, he developed sinusoidal obstructive syndrome and progressive liver failure treated with defibrotide and he was admitted to ICU for sepsis. Leucocyte count showed sufficient engraftment on day 14; however, liver function recovered only partially. During critical care treatment, the patient showed cardiomyopathy, renal failure and extensive wound healing problems without epithelial proliferation indicative of severe replicative exhaustion. Finally, he died due to sepsis with acute liver failure on day 91 after aSCT. AML arising from DKC is a rare event with substantial impact on patients' prognosis. Therapy remains challenging due to poor BM function and high risk of organ toxicity, especially liver failure and lung fibrosis. Dose reduction of alkylating agents and avoidance of total body irradiation are necessary in conditioning prior to aSCT in patients with DKC and AML, however no clear data or recommendations exist for the management of these patients. TL screening can help to identify patients with suspected DKC related BM failure or AML and to identify family donors without telomeropathy. Physicians should be aware of possible DKC related AML, especially in familial cases of AML or bone marrow failure, impaired or prolonged recovery following cytoreductive treatment or coincidence of solid (e.g. oral cavity carcinomas) and hematological malignancies.

**Disclosure of conflict of interest:** None.

#### P519

##### Chronic graft-versus-host disease and donor lymphocyte infusions in patients with non-de novo acute myeloid leukemia or advanced myelodysplastic syndromes after allogeneic stem cell transplantation

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AML with myelodysplasia-related changes and therapy-related AML (tAML), collectively termed secondary AML (sAML) in daily clinical routine, represent distinct subgroups in the 2016 revised WHO classification of myeloid neoplasm and leukemias. As compared to de novo-AML, sAML is associated with a poor survival when using conventional chemotherapy approaches. This is mainly due to unfavorable cytogenetics, older age and/or the presence of comorbidities as well as poor response to induction therapy. Furthermore, cumulative organ toxicity resulting from treatment of the antecedent solid malignancy in patients with therapy-related disease has to be taken into account. Allogeneic stem cell transplantation (alloSCT) represents the only option to achieve long-term disease control and definitive cure. We retrospectively analyzed 204 patients with sAML or advanced MDS (EB-2 according to WHO) transplanted at our center between 1995 and 2015. At the time of alloSCT, 98 patients (48%) were in complete hematologic remission (CHR), whereas 106 patients (52%) had active disease. Cytogenetic risk was categorized according to the SWOG/ECOG classification and was favorable (N=3; 2%), intermediate (N=94; 46%), unfavorable (N=84; 41%), or unknown/undetermined (N=23; 11%). Standard myeloablative conditioning (MAC) using 12 Gy total body irradiation (TBI) and cyclophosphamide was used in 41 patients (20%), whereas fludarabine/busulfan/ATG-based reduced intensity conditioning (RIC) was applied in 163 patients (80%). Grafts were from related (N=51; 25%) or unrelated (matched: N=112; 55% or mismatched: N=41; 20%) donors. The median follow-up of the surviving patients was 46 (5–24) months. A graft failure occurred in 5/204 patients (3%). At last day of follow-up 72/204 patients (35%) were alive and in CHR. Relapse occurred in 77/204 patients (38%) after a median interval of 4.6 (range: 0.1–135) months. Cause of death

were either relapse or NRM (GvHD and/or infections) in 69/204 patients (34%) or 56/204 patients (28%). At 1, 3, 5, and 10 years after alloSCT overall survival (OS) or disease-free survival (DFS) of the entire cohort was 56%, 46%, 38%, and 29% or 50%, 38%, 36%, and 27%, respectively. At the same time points, the cumulative incidence of relapse (CI-R) or non-relapse mortality (CI-NRM) was 30%, 37%, 37%, and 40% or 20%, 25%, 27%, and 33%, respectively. Extensive uni- and multivariate analyses revealed a number of factors associated with inferior outcome, e.g. poor-risk cytogenetics, the presence of tAML, advanced age, reduced physical performance, and comorbidities, whereas donor type (unrelated versus unrelated), and remission status had no significant impact on overall outcome. Furthermore, the development of GvHD, especially the presence of cGvHD, and the use of donor-lymphocyte infusions (DLI), either in a prophylactic or pre-emptive setting, were identified as independent predictors for a reduced relapse incidence, which in turn, led to an improved OS and DFS. Our results indicate that alloSCT represents an important treatment option for patients with sAML. However, a relapse rate of 30% at 12 months prompts the development of novel approaches to prevent early disease recurrence. Strategies to augment the graft-versus-leukemia (GvL) effect of alloSCT may help to improve the results.

**Disclosure of conflict of interest:** None.

#### P520

##### **Clinical outcome of myeloid sarcoma in adult patients and effect of allogeneic stem cell transplantation: results from a multicenter survey**

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Myeloid Sarcoma (MS) is a rare hematologic myeloid neoplasm that can involve any site of the body. It can occur as an exclusively extramedullary form or it can be associated with an acute myeloid leukemia (AML), a chronic myeloproliferative neoplasm (MPN) or a myelodysplastic syndrome (MDS) at onset or at relapse. The rarity of MS does not enable prospective clinical trials and therefore a specific multicenter register can be useful for the clinical and biological studies of this rare disease. We report the clinical characteristics and outcome of 48 histologically confirmed MS, diagnosed and treated in 9 Italian Hematological Centers in the last 10 years. The patient's median age was 46 years. There were 9/48 *de novo* extramedullary MS, 24/48 *de novo* AML-related MS and 15/48 were secondary AML-related MS. The most common extramedullary anatomic sites of disease were: skin, lymph nodes and soft tissues. Forty-three patients (90%) underwent a program of intensive chemotherapy including FLAI, HDAC-IDA, HyperCVAD and MEC schemes, with a CR Rate of 44% (19/43). Twenty-two (46%) patients underwent Allogeneic SCT, 13 from a MUD, 8 from an HLA-identical sibling donor and 1 from an haploidentical donor. The median OS of the whole population (48 pts) was 16.7 months. The OS probability at 1, 2 and 5 years was 64%, 39% and 33%, respectively. The OS was better

in patients that underwent an intensive therapeutic program (median OS: 18 months vs 5 months). Among the intensively treated patients, in univariate analysis, the OS was better in young patients ( $P=0.008$ ), in patients that underwent Allo-SCT ( $P=0.009$ ) and in patients that achieved a CR during treatment ( $P=0.001$ ), and was worse in pts with secondary AML-related MS ( $P=0.007$ ). Age, response to intensive chemotherapy and Allo-SCT were the only three variables that significantly influenced DFS ( $P=0.02$ ,  $P=0.01$  and  $P=0.04$ , respectively). In multivariable analysis, Allo-SCT and response to intensive chemotherapy remained significant in predicting a better OS ( $P=0.04$  and  $P=0.001$ , respectively), and response to intensive chemotherapy was the only significant variable in predicting DFS ( $P=0.01$ ). After Allo-SCT we observe a survival advantage in patients who achieved a pre-transplant CR ( $P=0.008$ ) and in those who developed a chronic GvHD ( $P=0.05$ ). Patients with MS, both with *de novo* and secondary forms, still have a very unfavorable outcome and require an intensive therapeutic program, that includes Allo-SCT, whenever possible. The outcome after Allo-SCT is positively influenced by the development of chronic GvHD suggesting a Graft versus MS effect.

**Disclosure of conflict of interest:** None.

#### P521

##### **Concurrent blinatumomab and donor lymphocytes for relapse of acute lymphoblastic leukemia after 2nd allogeneic transplantation—a case report**

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Relapse of acute lymphoblastic leukemia (ALL) after allogeneic stem cell transplantation (SCT) is associated with poor prognosis. Blinatumomab may enhance the efficacy of donor lymphocyte infusions (DLI) in this specific situation but data on the concurrent use of DLI and blinatumomab are sparse. The patient presented here was diagnosed with standard risk pre-B-ALL (presence of t(3;9); bcr-abl and CD20 negative) at the age of 23. During treatment according to the German Multicenter ALL-study group (GMALL) protocol he presented with molecular relapse and 8 months after initial diagnosis he received a TBI-based myeloablative SCT from an unrelated HLA-identical (10/10) donor. Post SCT he was negative for minimal residual disease (MRD) with 100% donor engraftment. Given the high relapse risk he received 3 prophylactic DLI without occurrence of graft-versus-host disease (GvHD). One year after 1st SCT he presented with an extramedullary (testes) and molecular relapse. After remission induction resulting in negative MRD he received a 2nd SCT from an alternative, HLA-identical (10/10) donor after reduced intensity conditioning. This again resulted in negative MRD with 100% donor chimerism without any GvHD. Six months after 2nd SCT he presented with bone marrow relapse. We decided on the concurrent use of blinatumomab and DLI. The first cycle of blinatumomab was initiated at standard dose including dose escalation without relevant toxicities. On day 40 of the 2nd cycle, i. e. in the infusion-free interval before the 3rd cycle the patient received the first DLI at  $1 \times 10^7$  CD3/kg. No toxicities or GvHD occurred. The 3rd cycle of blinatumomab was initiated and a second DLI at  $2.5 \times 10^7$  CD3/kg was applied on day 3 of the 3rd cycle. On day 32 of the 3rd cycle, i. e. day 29 after 2nd DLI the patient presented with signs of overlap GvHD (mouth, skin) and topical steroids were started. Upon progression of clinical GvHD systemic steroids were initiated with immediate response. Steroids were rapidly tapered and a 4th cycle of blinatumomab was started. GvHD did not recur. Current staging after the 4th cycle blinatumomab, i.e. on day +413 after 2nd SCT and 7 months after initiation of blinatumomab treatment revealed complete remission with negative MRD, 100% donor chimerism and no signs of extramedullary relapse. Counts of CD4-cells at that time point were 147/ $\mu$ l.

No relevant infections or relevant blinatumomab-associated toxicities were present during the entire course after the 2nd SCT. In this case concurrent treatment of blinatumomab and DLI resulted in the longest disease-free interval for our patient compared to preceding chemotherapy or DLI alone. Together with the small number of reported cases (Ueda et al.) this supports the concept of concurrent blinatumomab and DLI as an effective post SCT treatment.

#### Reference

1. Ueda et al. *Biol Bone Marrow Transplant* 2016; **51**:1253.

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#### P522

##### **Decitabine combined with HAAG regimen is an effective salvage treatment for advanced acute myeloid leukemia**

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The objective of the study is to evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen [homoharringtonine (HHT), cytarabine (Ara-C), doxorubicin (Acl) and recombinant human granulocyte colony stimulating factor (G-CSF)] for advanced patients with acute myeloid leukemia (AML). Thirty-six patients with advanced AML receiving DAC combined with HAAG chemotherapy in our center from December 2012 to August 2015 were enrolled in this study. Eighteen of them were refractory or relapsed AML, and another 18 patients were those who didn't achieve complete remission (CR) after a course of induction chemotherapy. The therapeutic responses, side effects and long-time survival were retrospectively analyzed. After a course of treatment, the rate of CR and partial response (PR) was 58.3% (21/36) and 22.2% (8/36) respectively, while the overall response rate (ORR) was 80.6% (29/36) in the cohort. For the patients with refractory or relapse AML, CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 4 hematological toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5 ~ 33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapsed AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly ( $P=0.01$ ). Conclusion DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.

**Disclosure of conflict of interest:** None.

#### P523

##### **Different pre-transplant conditions, different prognostic impact of FLT3-ITD mutation on the post-transplant survival: a report from Taiwan bone marrow transplant registry (TBMTR)**

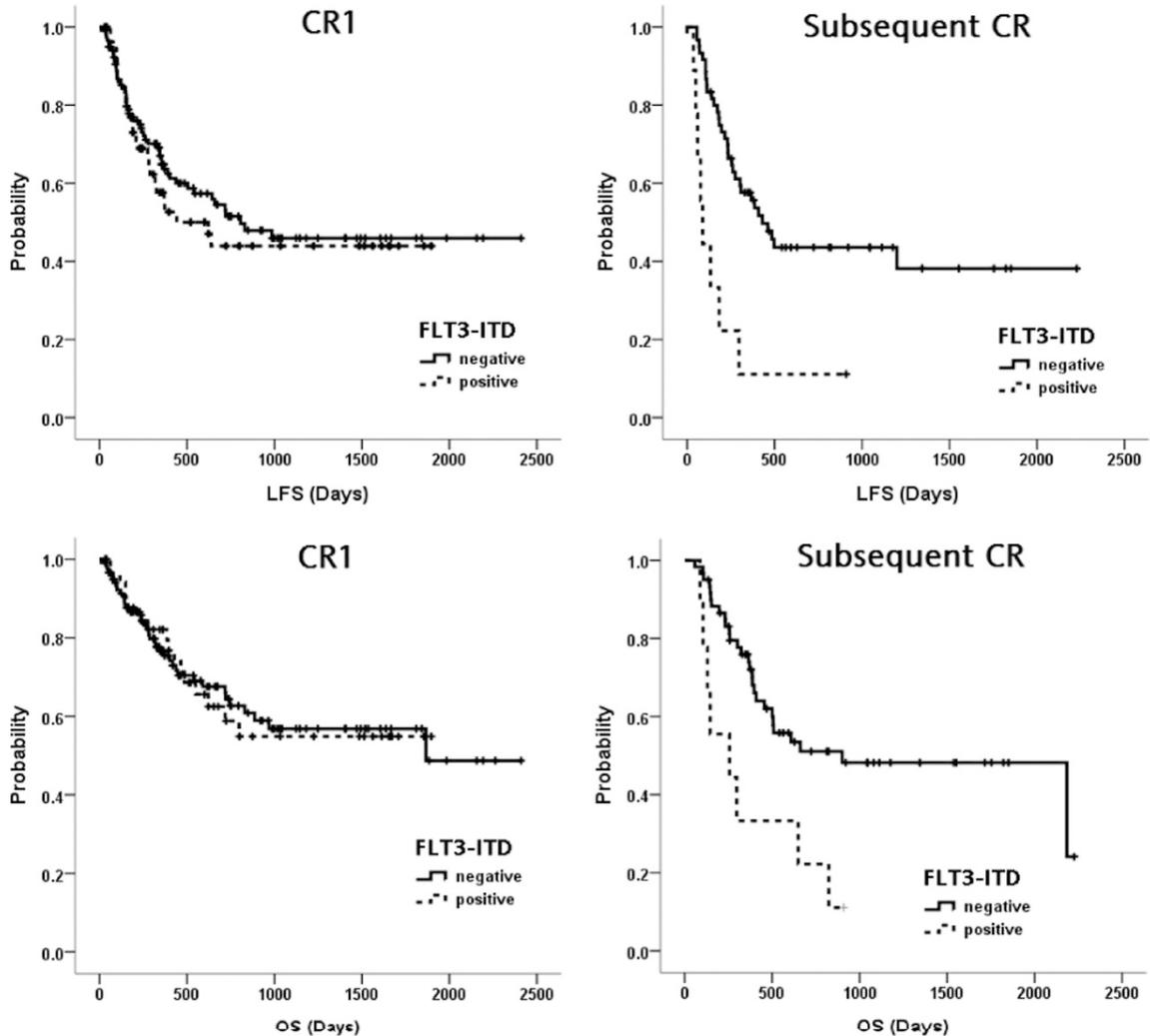
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AML patients harboring FLT3-ITD mutation are associated with decreased survival compared to patients without FLT3-ITD mutation. Nevertheless, whether FLT-ITD mutation also has negative impact on the post-transplant survival is less clear. For FLT3-ITD mutated AML, a decreased leukemia-free survival (LFS) after allogeneic HSCT was observed in EBMT analysis but not CIBMTR. In this study, unlike studies of EBMT or CIBMTR which only pre-specified populations of patients were analyzed (CR1 in EBMT, CR1+CR2 in CIBMTR), we examined the prognostic impact of FLT3-ITD mutation on post-transplant outcome of "all" the adult AML patients reported to Taiwan Bone Marrow Transplant Registry (TBMTR). TBMTR is a research collaboration affiliated to the Taiwan Society of Blood and Bone Marrow Transplantation. It comprises all the 14 transplantation centers in Taiwan that contribute detailed data on HSCT. Adults aged  $\geq 18$  years with a diagnosis of AML and with known FLT-ITD mutation status in the registry were included. Patient characteristics and transplant outcome following allogeneic HSCT for FLT3-ITD mutated and non-mutated AML were compared. Kaplan-Meier estimates were used to calculate the probability of LFS and overall survival (OS). Multivariable analyses for LFS and OS were performed using Cox proportional hazards model. 365 patients who met the eligibility criteria were enrolled for analysis. The median follow-up of survivors was 21 months. Of the 365 patients, 94 (25.8%) were positive and 271 (74.2%) were negative for FLT3-ITD mutation. FLT3-mutated patients had significantly more transplantation at CR1 (57.4%), shorter time interval between diagnosis and HSCT (5.6 months), and higher WBC count at diagnosis ( $51.7 \times 10^9/L$ ) comparing to patients without FLT3 mutation (43.5% at CR1, 6.5 months from diagnosis to HSCT, and  $11.8 \times 10^9/L$  WBC count at diagnosis). Significant more FLT3 mutated patients had intermediate-risk (80.9%) and normal (64.9%) karyotype at diagnosis. The age, donor type, stem cell source, conditioning regimen, and ATG use were not significant different between FLT3-mutated and non-mutated patients. Of the whole population, FLT3 mutation status did not negatively impact the transplant outcome (2 years OS for FLT3 mutated and non-mutated patients: 45.2% vs 50%, log rank  $P=0.624$ ; 2 years LFS for FLT3 mutated and non-mutated patients: 40.2% vs 32.4%, log rank  $P=0.192$ ). When different pre-transplant conditions (CR1, subsequent CR, and no CR) were analyzed separately, FLT3-ITD mutation status is still not a significant prognostic factor of OS and LFS for patients in CR1 (equally good) and no CR (equally bad). However, for patients in subsequent CR, FLT3-ITD mutation is the only significant factor predicting poor OS and LFS in multi-variable analysis (median OS and LFS for FLT3 mutated and non-mutated patients: 378 vs 1252 days, log rank  $P=0.005$ ; 204 vs 1049 days, log rank  $P < 0.001$  respectively). The incidence of non-relapse mortality, grade 3/4 acute GVHD and extensive chronic GVHD is comparable between FLT3-mutated and non-mutated patients. FLT3-ITD mutation is a significant and strong predictor of poor survival for AML patients in subsequent CR at HSCT. For FLT3-ITD non-mutated AML, a sizable portion of patients can have disease free survival after allogeneic HSCT at subsequent CR. However, allogeneic HSCT at CR1 should be strongly recommended for FLT3-ITD mutated AML.

[P523]



**Disclosure of conflict of interest:** None.

**P524**

**Extramedullar relapse after allogeneic stem cell transplant in myeloid malignancies: A center experience**

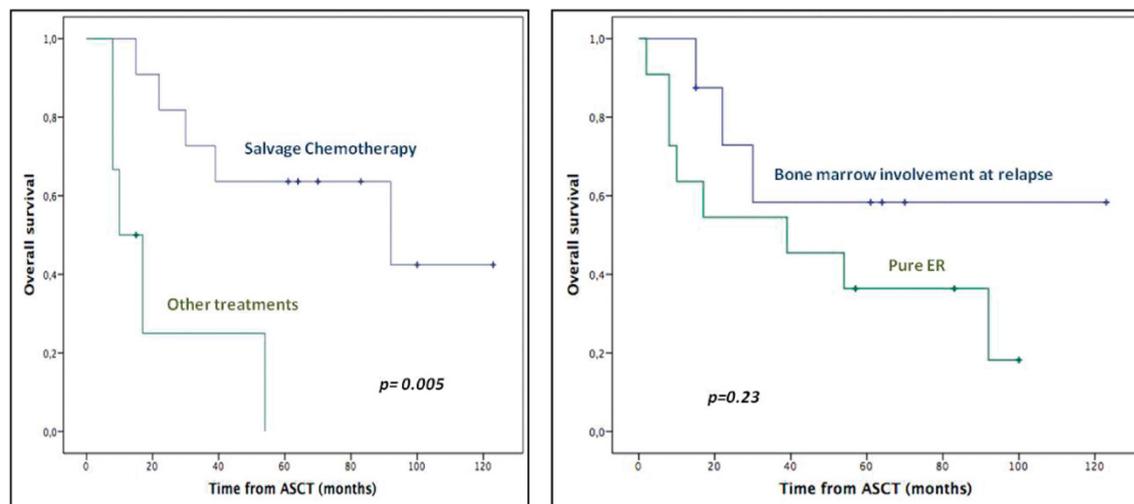
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Allogeneic stem cell transplantation (ASCT) is a curative strategy in acute myeloblastic leukemia (AML) and myelodysplastic syndrome (MDS). However, relapse keeps being the main cause of treatment failure. Extramedullary relapse (ER) is a rare event and its management is not well standardized. We retrospectively analyzed patients who received ASCT from 2006 to 2016 and developed ER in our centre. We performed a descriptive study to analyze characteristic of these patients, post-relapse treatment and survival. Statistic analysis was performed using SPSS v.22. We found a total of 18 patients with ER, one of them with 2 ER after 2 consecutive ASCT, so we analyzed 19 cases of ER. Patient and transplant characteristics are summarized in Table 1. At day +100, 95% of patients were in complete response (CR). ER occurred after a median of

13 (2–98) months post-ASCT. Eleven patients (58%) presented with a bone marrow relapse concomitant with the ER. ER affected central nervous system (CNS) in 7 patients (36.8%), bone in 4 patients (21%), skin or soft tissue in 3 patients (15.8%), mama in 2 patients (10.5%), ocular globe in 2 patients (10.5%) and teste in 2 patients (10.5%). Two of them presented with multiple sites affected. Between the 7 patients who developed CNS relapse, 2 of them had received intrathecal prophylaxis. Regarding post-ER management, immune modulation was conducted in 16 patients (immunosuppression tapering in 9, donor lymphocyte infusions in 4 and both strategies in 3). All patients except one received systemic treatment (salvage chemotherapy in 11, azacitidine in 5, low dose AraC in 1 and ATRA in 1 patient with a promyelocytic leukemia). Together with systemic treatment, 12 received radiotherapy and intrathecal therapy was used in all 7 patients with CNS involvement. Response: 12 out 18 patients treated, 12 (63.2%) achieved CR and 6 (31.6%) progressed. Two responding patients received a 2<sup>nd</sup> ASCT. After a median follow-up of 67 months (15–123), 8 patients are alive and disease free, with an estimated overall survival of 45% at 5 years. Patients receiving salvage chemotherapy followed or not by a 2<sup>nd</sup> ASCT experienced a significantly better OS than those receiving other therapies (median OS 92 vs 10 months;  $P=0.005$ ). Patients with bone marrow involvement at relapse show a worse prognosis (median OS 39 vs 54 months;  $P=0.23$ ) although not statistically significant due to small number of patients (Image 1). Ten patients died due to disease progression. ER must be

[P524]



considered in patients receiving an ASCT in case of organ symptoms. Patients can be rescued with salvage chemotherapy followed or not by a 2<sup>nd</sup> ASCT achieving good results in terms of long term OS. It seems that involvement of bone marrow at relapse confers a worse prognosis, what should be confirmed in a larger series of patients.

[P524]

Table 1

Patient characteristics (n=18)	
Age at diagnosis (years), median (range)	43.50 (24-62)
Female (%)	12 (66.7%)
Diagnosis	
AML (%)	17 (94.4%)
MDS (%)	1 (5.6%)
Prior extramedullary disease (%)	2 (11.1%) (Skin and CNS infiltration)
Hyperleukocytosis at diagnosis (%)	8 (44.4%)
FAB M4/M5 (%)	3 (16.7%)
FLT3+ (%)	4 (22.2%)
CD56 expression (%)	7 (38.9%)
Transplant characteristics (n=19)	
Disease status at transplantation (%)	
Complete response (%)	14 (73.7%)
Other (%)	5 (26.3%)

**Disclosure of conflict of interest:** None.

## P525

### FLAG-IDA regimen as bridge therapy to allotransplant in refractory/relapsed aml patients: a single-center experience

C Pasciolla, M Delia, D Pastore, P Carluccio, A Ricco, A Russo Rossi, A Mestice, F Albano and G Specchia  
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Although treatment outcome in acute myeloid leukemia (AML) adult patient has improved over the past decade, relapse still occurs in up to 50–70% of cases. Furthermore, 15–30% of patients fail to achieve complete remission (CR) because of treatment-resistance. The management of primary refractory and/or relapsed disease remains challenging for clinicians. In our study, we reviewed the outcome of 116 refractory and/or relapsed AML patients who underwent salvage therapy with the FLAG-Ida regimen between 2005 and 2015 at our institution. The study aim was to determine the efficacy of

the FLAG-Ida regimen in order to clarify which variables (WHO PS, LDH, bone marrow, peripheral blood blasts and platelets counts, white blood cells (WBC), PMN, molecular-cytogenetic risk, duration of response and relapsed or refractory disease), present before starting FLAG-Ida treatment, might have an impact both on CR and on OS. We analyzed 116 consecutive adult patients (56 males, 60 females; median age 48 years, range 17–72) with newly diagnosed acute myeloid leukemia refractory to standard induction regimens or relapsed after CR, who received the FLAG-Ida protocol as salvage therapy between January 2005 and December 2015. Sixty-eight of the 116 patients (58%) were in first relapse, forty-seven patients (42%) were refractory to conventional chemotherapy. Median WBC count before salvage therapy was 10.1 x10<sup>9</sup>/l (range 0.56–88). Median bone marrow and peripheral blasts counts were 52 and 20%, respectively; median platelets count was 91x10<sup>3</sup>/μL. According to the FAB classification, 14 patients had M0, 5 M1, 53 M2, 16 M4, 22 M5, 4 M6, 2 had Biphenotypic Acute Leukemia. According to molecular-cytogenetic risk stratification 51 (44%), 44 (38%) and 21 (18%) patients belonged to poor, intermediate and good risk group, respectively. Sixty-nine of 116 patients (59%) achieved complete remission (CR); forty-seven (41%) patients were refractory to the salvage therapy. In multivariable analysis, variables with positive impact on response rate were lower WBC counts (< 10e<sup>3</sup>/μL,  $P=0.0047$ ), higher platelets counts (> 50x10<sup>3</sup>/μL,  $P=0.046$ ), molecular-cytogenetic risk ( $P=0.032$ ), duration of response in relapsed AML ( $P=0.006$ ) and relapsed rather than primary refractory disease ( $P=0.042$ ), respectively. Median OS was 17 months (m). Cox regression analysis confirmed that both higher platelets counts,  $P=0.002$  (17 (> 50x10<sup>3</sup>/μL) vs 11 m (< 50x10<sup>3</sup>/μL), log Rank,  $P=0.05$ ) and relapsed disease,  $P=0.041$  (23 (relapsed) vs 17 m (refractory), Gehan-Breslow,  $P=0.021$ ) correlated with better survival. Of note, molecular-cytogenetic risk evaluated before starting treatment was associated with CR, while no correlation was found with OS. Our data seem to confirm the value of FLAG-Ida in relapsed AML and may suggest its best usage as bridge-therapy in patients awaiting allotransplantation.

**Disclosure of conflict of interest:** None.

## P526

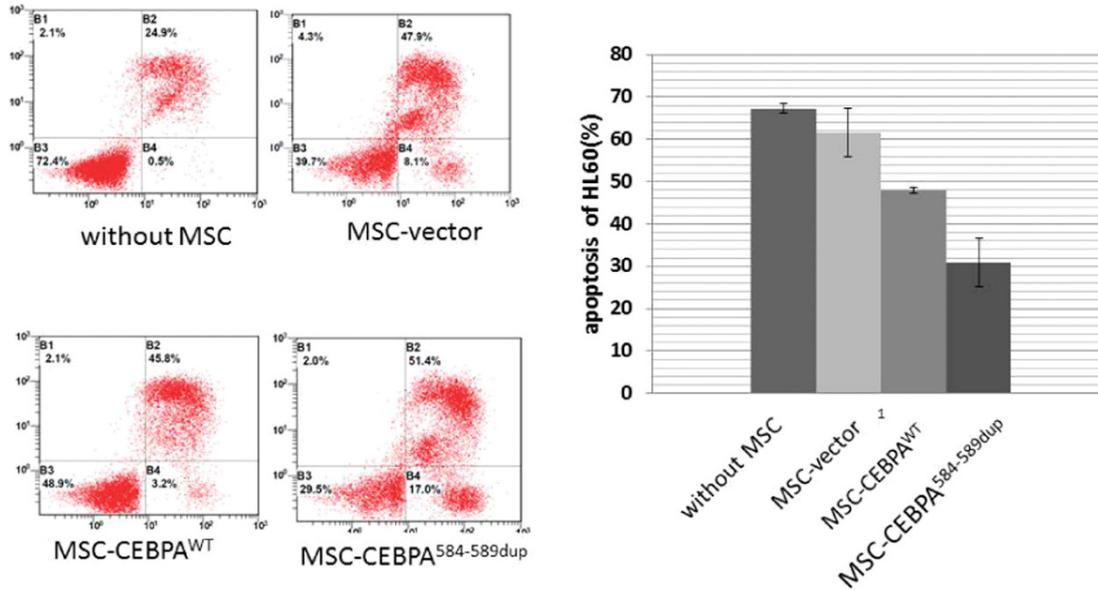
### Germ line c.584-589dup mutation of CEBPA gene strengthens bone marrow niche and increases the survival of AML cells under chemotherapy

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Leukemia relapse is the major cause of death in patients received allogeneic hematopoietic stem cell transplantation (allo-HSCT). The precise etiological mechanisms of leukemia relapse remain unclear. Both leukemia cells themselves and hematogenesis micro-environment are involved in the relapse event. In our previous study, we reported a case of donor derived relapse of acute myeloid leukemia (AML) after allo-HSCT. The patient and his donor-sister both harbored a germline mutation(c.584-589dup) in CEBPA gene. Donor hematopoietic cells transformed to AML by developing two somatic CEBPA mutations (247dupC and 914-916dup) in the patient's microenvironment. Hence we suspect that 584-589dup

mutation of CEBPA gene may altered hematopoiesis micro-environment and increased the survival of AML cells. To conform our hypothesis, we transfected mesenchyme stem cells (MSCs) with CEBPA<sup>584-589dup</sup> or wide type and took vector as control. AML cell line HL60 cells were co-cultured with transfected MSCs and then treated with 40ng/ml Doxorubicin. Apoptosis and cell cycle were detected at day 3. MSCs protected HL60 cells from toxicity of Doxorubicin. This protection was enhanced by overexpression of CEBPA<sup>584-589dup</sup>. Apoptosis rates of HL60 cells in group of MSC-vector and MSC-CEBPA<sup>584-589dup</sup> were 61.7 ± 5.8% vs 30.9 ± 5.6% (p < 0.05). A larger part of HL60 cells remains quiescent with

[P526]



Figur 1. Apoptosis of HL60 cells was detected after co-culture with MSC and 3 days treatment of Doxorubin(40ng/ml) .

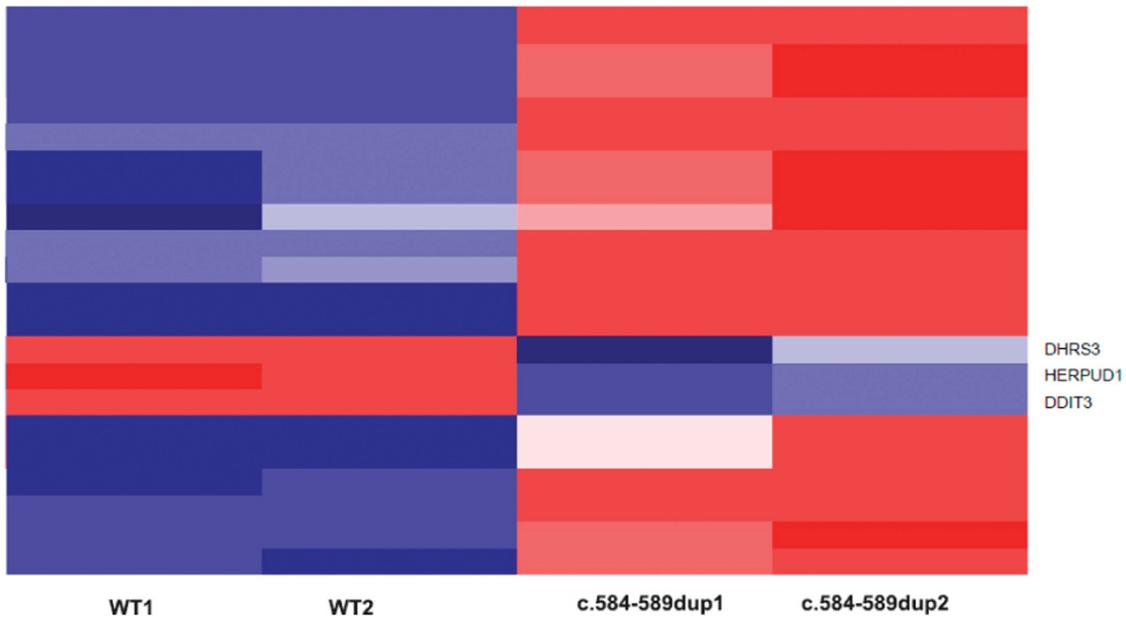


Figure 2. Heatmap of unfolded protein response(UPR) related genes in MSCs cells, detected by RNA-seq.

higher rate of G0/G1 phase in MSC-CEBPA<sup>584-589dup</sup> group, which may reduce the sensibility of HL60 cells to Doxorubicin. To explore mechanisms involved in the alteration of micro-environment, we performed RNA sequence with each group of MSCs. We found that COL1A1, COL1A2 and COL3A1 were upregulated in MSC-CEBPA<sup>584-589dup</sup> group compared with MSC-CEBPA<sup>WT</sup> group (COL1A1:CEBPA<sup>WT</sup> vs CEBPA<sup>584-589dup</sup> was 1713.65 vs 2317.88,  $P=4.70E-19$ ; COL1A2:CEBPA<sup>WT</sup> vs CEBPA<sup>584-589dup</sup> was 2260.02 vs 2755.81,  $P=2.76E-10$ ; COL3A1:CEBPA<sup>WT</sup> vs CEBPA<sup>584-589dup</sup> was 746.20 vs 964.82,  $P=1.06E-06$ ). Furthermore, we found that DDIT3 and HERPUD1 genes, which were important factors in cellular unfolded protein response (UPR) and to topologically incorrect protein, failed to augment in CEBPA<sup>584-589dup</sup> group (DDIT3: vector vs CEBPA<sup>WT</sup> was 25.04 vs 56.36 ( $P=0.00037$ ), vector vs CEBPA<sup>584-589dup</sup> was 25.04 vs 17.6917 ( $P=0.25$ ), HERPUD1: vector vs CEBPA<sup>WT</sup> was 46.93 vs 90.40 ( $P=0.00014$ ), vector vs CEBPA<sup>584-589dup</sup> was 46.93 vs 41.07 ( $P=0.503$ )). Upregulation of supportive genes and impaired UPR-triggered apoptosis may strengthen bone marrow niche for transformed blood cells collaboratively, thereby help leukemia cells escaping from toxicity of chemotherapy.

**Disclosure of conflict of interest:** None.

## P527

### Previously published

## P528

### Hematopoietic stem cell transplantation for childhood leukemia in Mahak

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The cure rate of childhood acute lymphoblastic leukemia (ALL) has improved considerably and approaches 80% today. However, the outcomes of patients who suffer from leukemic relapse remain unsatisfactory. Despite the high cure rate of children and adolescents with ALL a subgroup of patients benefit from allogeneic HSCT. Allo HSCT remains the standard treatment for intermediate/high risk AML patients. 59 patients, ALL = 42 and AML = 17 age 1 to 20 years with median age 11 years, M/F = 33/26 (M/F ALL = 15/18, AML = 8/9) underwent SCT in our hospital (from 2012 to 2016). Fifty-eight patients transplanted Allo HSCT and 1pt AML Auto HSCT. Conditioning regimens consisted of Busulfan (IV) + Cyclophosphamide for Allo and Cyclophosphamide + VP16 + Cytarabine for Auto HSCT. Peripheral blood (PB) was the source of progenitor cells in 47 patients, Bone marrow (BM) in 11 patients and cord blood in one patient. In Allo HSCT, 50 patient transplanted 6/6 matched and 8 patients 5/6 matched. GVHD prophylaxis regimen was cyclosporine + Mtx. All patients engrafted. In allogeneic PBSCT ALL patients' median time to absolute neutrophil count (ANC)  $>0.5 \times 10^9/L$  was 12 days, and the median time to platelet count  $>20 \times 10^9$  was 15 days vs 17 and 21 days in Allo BM ALL patients. In allogeneic PBSCT AML patients median time to ANC  $>0.5 \times 10^9/L$  was 12 days, and the median time to platelet count  $>20 \times 10^9$  was 14 days. (All patients with AML transplanted with PB). At present 47 pts are alive (36 ALL, 11 AML) and 12 pts died due to ARDS, VOD, hemorrhagic stroke, sepsis and relapse. TRM was 9% at 100 days. Median time of death after transplantation was 195 days in ALL and 153 in AML. In Allo PBSCT ALL patients hospitalization period were 36 days vs 45 in Allo BM ALL patients. Acute GVHD appeared in 78% pts. Chronic GVHD appeared in 55% pts. With a median follow-up of 35 months (3–51 months) after transplant the event-free survival were 73% and four years overall survival 75% in ALL patients. A median follow-up of 32.5 months (4–48 months) after transplant the event-free survival were 68% and three years overall

survival 63% in AML patients. Hematopoietic stem cell transplantation can lead to durable remissions in children and adolescents with leukemia and increase in survival of children. PBSCT in childhood ALL was consistent with significant faster ANC and platelet recovery in allogeneic PBSCT, hospitalization was shorter. Longer follow-up is required to evaluate fully efficacy and long-term results.

**Disclosure of conflict of interest:** None.

## P529

### HLA C1/C1 homozygosity decreases relapse rate after allogeneic hematopoietic stem cell transplantation (Allo-HSCT) in acute myeloid leukemia (AML) patients

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Natural killer cells (NK) demonstrate anti-leukemic activity by activating or inhibiting killer cell immunoglobulin-like (KIR) receptors. KIR receptors characterized in MHC class I ligands are HLA-C, HLA-B-Bw, HLA-A and HLA-G. HLA-C divides into two subtypes, HLA-C1 and C2 due to amino acid residues. Recently, studies showed that presence of HLA-C2 in childhood acute lymphoblastic leukemia (ALL) predispose to disease and increase risk of late relapse (Babor et al, Blood 2014). A KIR2DS1 dependent graft-versus leukemia effect reduced the risk of relapse in AML however the benefit was shown to be eliminated in HLA-C2/C2 homozygote patients (Venstrom et al, NEJM 2012). In this study, we aim to demonstrate the effect of HLA-C subtype in overall survival (OS), risk of relapse, leukemia free survival (LFS) in patients with AML. The study included 122 AML patients who underwent allo-HSCT in Ankara University School of Medicine Department of Hematology ( $n=89$ ), Anadolu Health Center ( $n=14$ ), Florence Nightingale Hospital ( $n=6$ ), Goztepe Medical Park Hospital ( $n=5$ ), Medipol Hospital ( $n=3$ ), Antalya Medical Park Hospital ( $n=3$ ), Bahcelievler Medical Park Hospital ( $n=2$ ) between 2007 and 2016. HLA Class I typing was performed by Luminex PCR-SSOP method (Invitrogen and One Lambda). Group HLA-C1/C2 subtypes were defined as previously practiced. Median age of patients was 41, 47% of them were male. Allo-HSCTs were performed from 60% unrelated donors vs 40% related donors. Remission status was detected in 67% of patients whereas 12% had active disease pre-transplant. Stem cell sources were as follows: 88% peripheral blood, 7% bone marrow, 3% cord blood, 2% bone marrow plus peripheral blood. The most frequent FAB subtype was AML-M4. Patients were grouped by HLA-C status: 23% C1/C1 homozygote, 57% C1/C2 heterozygote and 20% C2/C2 homozygote. The frequency of HLA C donor/recipient mismatch allo-HSCTs was 19%. Relapse was detected in 26% of patients. The relapse risk was significantly lower in C1/C1 homozygote patients compared to C2/C2 homozygotes (13% vs 36%,  $P=0.02$ ). LFS was similar between C1/C1 homozygote group and C2/C2 homozygote group ( $P=0.324$ ) (Figure 1). In multivariate analysis (age, sex, remission status, related/unrelated transplant, AML subtype), LFS was increased by pre-transplant remission status (p1 year). There was no difference detected between 2-years OS in C1/C1 homozygote group and other groups (57% vs 50%,  $P=0.51$ ) (Figure 2). When similar analysis were repeated with donor HLA type results were not significant. Our results confirm two earlier published reports on AML and ALL. Even in the absence of KIR genotyping, HLA Group C1 has a protective effect. If HLA matched donor is not possible a donor-recipient HLA-C mismatch favoring C1 to C2 may be preferable.

[P529]

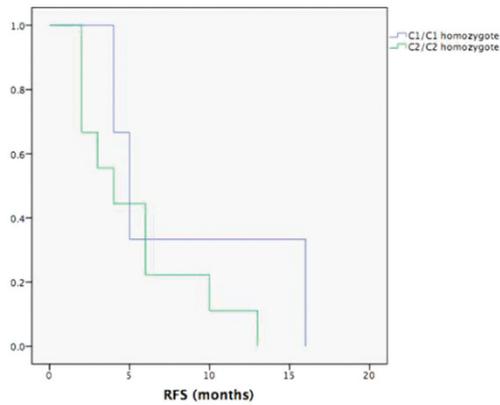


Figure 1. RFS between groups

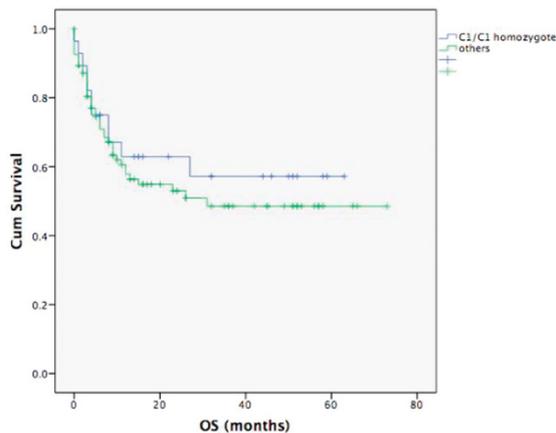


Figure 2. OS between groups

**Disclosure of conflict of interest:** None.

**P530**

**Immunomodulatory Kits do not induce AML-blasts' proliferation ex vivo: IPO-38 is an appropriate and reliable marker to detect and quantify proliferating blasts**

C Plett, DC Amberger, A Rabe, D Deen, Z Stankova, A Hirn, Y Vokac, J-O Werner, J Schmohl, D Krämer, A Rank, C Schmid and H Schmetzer

AML-blasts can be converted to DCleu by immunomodulatory 'Kits' (*ex vivo*). T-cells' energy can be overcome after stimulation with DC/DCleu and results in antileukemic reactivity. A potential induction of blast-proliferation (e.g. by immunomodulatory Kit-application *in vivo*) in AML-pts has to be excluded. 8 Kits containing combinations of GM-CSF with 1–2 additional factors (PGE-1/2, Picibanil, IFN $\alpha$ , TNF $\alpha$ , Calcimionophore) were studied with respect to the generation of DC/DCleu from blasts, mediation of antileukemic reactivity (after DC/DCleu-stimulation) and with respect to their potential to induce blast-proliferation in a whole blood (WB) culture-system. We studied 3 different markers (IPO-38, Ki-67, CD71) and quantified blast proliferation before/after culture. We correlated findings with (*ex vivo*) antileukemic functionality, with disease-entities and the course of the disease. DC-generation: We could generate DC/DCleu regularly from WB culture from 36 AML-pts. Detection of blast proliferation: 65.6% (range 46–82%) of uncultured blasts expressed IPO-38, 33.1% (16–50%) CD71, 25.4% (8–43%) Ki-67. Induction of blast proliferation: Pooling all results we found lowest amounts of

proliferating blasts after culture with Kit I (GM-CSF+Picibanil, 10%  $\pm$  13.32), Kit K (GM-CSF+PGE2, 9.14%  $\pm$  12.01), Kit M (GM-CSF+PGE1, 7.67%  $\pm$  11.79). Amounts of proliferating blasts were lower compared to uncultured cells. Highest expression of proliferating blasts was found with IPO-38 followed by CD71 and Ki-67. We found few individual AML-samples with increased blast-proliferation after *ex vivo* Kit-culture. Antileukemic activity: T-cells stimulated with DCleu (generated with Kits) improved antileukemic activity. Correlations between blast-proliferation and antileukemic activity will be presented. Clinical correlations: Pts with bad (vs good) cytogenetic risk were characterized by higher proportions of proliferating blasts in uncultured blasts; in some Pts with iron-deficiency anemia (IDA) proportions of CD71+uncultured blasts were lower than of IPO-38/Ki-67+ blasts. IPO-38 is a stable marker to be used to quantify proliferating blasts in AML-pts. CD71 is also a good marker, although not suitable for some pts with IDA, Ki-67 is no reliable marker for every given pt. Subtypes of pts correlated with proportions of proliferating blasts. In general KIT treatment of blasts did only exceptionally induce blast proliferation *ex vivo*. In general lowest risk for blast proliferation was seen after culture with KIT I, K and M. T-cell-stimulation with DC/DCleu generated after Kit-treatment resulted regularly in antileukemic reactivity. We conclude that an *in vivo* treatment of AML-pts with Kits I, K or M might be safe (no induction of blast proliferation).

**Disclosure of conflict of interest:** None.

**P531**

**Impact of additional cytogenetic abnormalities in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation**

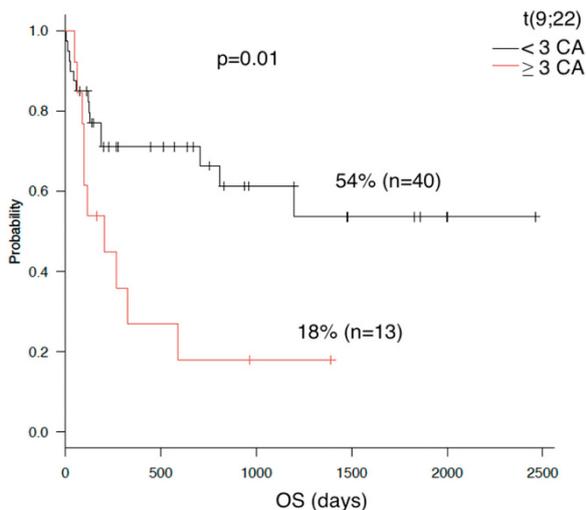
T Gindina, N Mamaev, A Alyanskiy, O Pirogova, Y Gudozhnikova, O Paina, P Kozhokar, E Nikolaeva, I Petrova, L Zubarovskaya and B Afanasyev

R.M. Gorbacheva Memorial Research Institute of Pediatric Oncology, Hematology and Transplantation at Pavlov First Saint Petersburg State Medical University

The occurrence of additional cytogenetic abnormalities (ACAs) is common in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), but is of unknown significance in the tyrosine kinase inhibitor era. Recent study [Aldoss *et al.*, 2015] has revealed the ACAs appear to have a significant deleterious effect on outcomes post-HSCT in adult Ph+ ALL patients only. We retrospectively analyzed data from adult and pediatric patients with Ph+ ALL who had undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) at our University between 2008 and 2015. Among 65 patients with Ph+ ALL, 53 patients had available data on conventional cytogenetics before allo-HSCT. All patients and transplant characteristics are listed in Table I. Thirty-three of 53 patients (51%) had isolated t(9;22). ACAs were revealed in 20/53 (31%) pts, including 13/53 (20%) pts with  $\geq 3$  cytogenetic abnormalities (with complex karyotype, CK). The median follow-up was 645 (26–2461) days. Overall survival (OS) and event free survival (EFS) were 48% (95% CI 7–33) and 30% (95% CI 17–44) at 4 years, respectively. In univariate analysis, prognostic factors associated with increased OS and EFS were donor type (match related/match unrelated vs haploidentical;  $P=0.02$  for both), the disease status at transplant (CR1 vs beyond CR1;  $P=0.01$ , only for EFS), ACAs (ACA- vs ACA+;  $P=0.04$ , only for OS) and, especially, the complex karyotype (CK- vs CK+;  $P=0.01$ , only for OS) (Figure 1). Multivariate analysis showed that the independent prognostic factors for OS and EFS remained the complex karyotype (HR-2.79, 95% CI, 1.23–6.34;  $P=0.01$ ) and disease status at transplant (HR-2.15, 95% CI, 1.13–4.09;  $P=0.01$ ), respectively. The study demonstrates the ACAs and disease status at allo-HSCT to be independent prognostic factors not only for adult, but for pediatric Ph+ ALL patients too.

**Table 1. Patient and Transplant Characteristics**

Number of patients	65 (100%)
<b>Patient sex, n (%)</b>	
Male	39 (60%)
Female	26 (40%)
<b>Age at HSCT, median, (range) years</b>	26.2 (5-48)
<b>Age group</b>	
≤18 yo	19 (29%)
≥18 yo	46 (71%)
<b>Cytogenetics, n (%)</b>	
t(9;22) without additional cytogenetic aberration	33 (51%)
t(9;22) with additional cytogenetic aberration	20 (31%)
Complex karyotype -	40 (62%)
Complex karyotype +	13 (20%)
No conventional cytogenetics	12 (18%)
<b>Clinical stage at HSCT, n (%)</b>	
CR1	31 (48%)
CR2	20 (31%)
Active disease	14 (21%)
<b>HSC source, n (%)</b>	
Bone marrow	31 (48%)
Peripheral blood	32 (49%)
Both	2 (3%)
<b>Conditioning regimen, n (%)</b>	
MA	29 (45%)
Non-MA	36 (55%)
<b>Donor type, n (%)</b>	
HLA-id sibling	18 (28%)
Matched unrelated	42 (64%)
Haploidentical	5 (8%)



**Disclosure of conflict of interest:** None.

**P532**  
**Previously published**

**P533**  
**Long term impact of hyperleukocytosis in newly diagnosed AML patients undergoing allogeneic stem cell transplantation in the era of advanced molecular prognostication**

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Up to 20% of newly diagnosed acute myeloid leukemia (AML) patients (pts) present initially with hyperleukocytosis consequently placing them at increased risk for morbidity and mortality during induction.<sup>1,2</sup> Whereas early publications<sup>3</sup> have indicated that hyperleukocytosis is an adverse prognostic factor associated with poor long term outcome, it is currently unknown whether hyperleukocytosis still retains prognostic value for AML patients undergoing allogeneic stem cell transplantation. Furthermore, it is unknown whether hyperleukocytosis retains prognostic value when modern molecular markers such as FLT3 and NPM1 are accounted for. We hypothesized that hyperleukocytosis at initial diagnosis is still an independent adverse prognostic factor influencing long term outcome in AML pts undergoing allogeneic stem cell transplantation. We performed a retrospective analysis using the multicenter registry of the acute leukemia working party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). Pts included in the analysis were over 18 years of age, with de-novo non-M3 AML, a presenting white blood cell count of over 100K, with an HLA matched related or unrelated donor, transplanted between 2005 and 2014. Clinical outcome indices of hyperleukocytosis pts namely, non-relapse mortality (NRM), graft versus host disease (GVHD), relapse incidence (RI), leukemia free survival (LFS), overall survival (OS) and GVHD-free/relapse-free survival (GRFS) were compared to a cohort of pts without presenting leukocytosis. Multivariate analyses were used to assess whether hyperleukocytosis was independently associated with RI, NRM, OS, LFS, and GRFS. Age, gender, number of chemotherapy inductions, cytogenetics, donor type, FMS-like tyrosine kinase-3 (FLT3) status, nucleophosmin (NPM1) status, and conditioning intensity were covariates for regression modeling. A cohort of 357 pts with hyperleukocytosis (159 patients with WBC over 50K and less than 100K, and 198 patients with WBC over 100K) was compared to 918 pts without hyperleukocytosis. Pts with hyperleukocytosis were younger, had an increased rate of favorable risk cytogenetics, were more likely to be FLT3 and NPM1 mutated, and had an increased rate of myeloablative conditioning. On univariate analysis pts with hyperleukocytosis had an increased rate of RI (30% vs 22.7%,  $P=0.013$ ), and decreased incidence of GRFS (36.6% vs 45.3%,  $P=0.022$ ). In multivariate regression analysis, hyperleukocytosis was significantly associated with increased RI (hazard ratio [HR] of 1.55, 95% confidence interval [CI], 1.145–2.124;  $P=0.004$ ),

poorer LFS (HR of 1.38, 95% CI, 1.071–1.785;  $P=0.013$ ), decreased GRFS (HR of 1.38, 95% CI, 1.117–1.71;  $P=0.002$ ), and poorer OS (figure 1) (HR of 1.4, 95% CI, 1.073–1.846;  $P=0.013$ ). Image/Graph Hyperleukocytosis at initial presentation retains a significant prognostic role for AML patients undergoing allogeneic stem cell transplantation even in the current era of advanced molecular prognostication.

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**Disclosure of conflict of interest:** None.

#### P534

##### Number, composition and/or antileukemic activity of (DC-stimulated) invariant NKT-, NK- and CIK-cells is predictive for outcome of patients with AML, ALL and CLL

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iNKT-/NK-/CIK-cell (subsets) are important for immune-surveillance. Antibody 6B11 targets the Va24-Ja18-invariant-T-cell-receptor (TCR) in the CDR3-region, which is semi-invariantly rearranged in iNKT-cells. We characterized: I.) iNKT-/NK-/CIK-subsets in PB-samples from healthy donors ( $n=9$ ), AML-pts( $n=23$ ), ALL-pts( $n=20$ ) and CLL-pts( $n=21$ ) in acute disease-stages and correlated findings with prognosis; II.) iNKT-/NK-/CIK-subsets under stimulation with Dendritic-Cells of leukemic origin (DC<sub>leu</sub>), generated from AML-blasts in mononuclear cells(MNC) and whole-blood (WB, containing soluble/cellular components of pts' PB) with 'cocktails' (DC-generating-methods/Kits). 1.1) Compared to healthy MNC (significantly) lower proportions of iNKT-cells (6B11<sup>+</sup>/6B11<sup>+</sup>CD3<sup>+</sup>/6B11<sup>+</sup>CD161<sup>+</sup>), NK-cells(CD3<sup>+</sup>CD56<sup>+</sup>/CD3<sup>+</sup>CD161<sup>+</sup>) and CIK-cells (CD3<sup>+</sup>CD56<sup>+</sup>/CD3<sup>+</sup>CD161<sup>+</sup>) were found in MNC from AML-/ALL-/CLL-pts. 1.2) Subtyping of iNKT-cells revealed (significantly) higher proportions of CD3<sup>+</sup>T-cells and CD161<sup>+</sup> NK-cells in AML-/ALL-/CLL expressing 6B11 compared to healthy MNC. 1.3) Prognostic evaluations showed higher proportions of iNKT-/NK-/CIK-cells in prognostically favorable AML-subgroups (allocation to younger age, primary disease-status, no extramedullary disease, achievement and maintenance of CR after induction-chemotherapy). Comparable correlations were seen in adult ALL- and CLL-pts. 2.1) We quantified iNKT-/NK-/CIK-subsets before/after mixed-lymphocyte-cultures (MLC) of T-cell-enriched immune-reactive cells stimulated with MNC/WB (with or without pretreatment 'cocktails' inducing blasts' conversion to DC<sub>leu</sub>) from AML-pts. Our findings show, that 1) iNKT-/NK-/CIK-cells increase after MLC independent of the stimulator-cells-suspension (under the influence of IL-2); 2) Pretreatment of MNC/WB-blasts with 'cocktails' increases iNKT-counts and induces a shift in the composition of iNKT-/NK-/CIK-subsets after MLC, that might correlate with an improved antileukemic potential; 3) INDIVIDUAL samples showed varying, however higher iNKT-, CIK-cell-counts after pretreatment with different (especially prostaglandin-containing) 'cocktails'; 4) DC-/iNKT-/NK-/CIK-cells-values after MLC were comparable in physiological hypoxia vs normoxia; 5) In cases with antileukemic blast-lytic activity after MLC T-/iNKT-/NK-/CIK-cells were significantly increased-pointing to an involvement of these cells in antileukemic reactions. In summary: (1) Healthy MNC present with significantly higher iNKT-/NK-/CIK-cells compared to AML-/ALL-/CLL-leukemic MNC. (2) Subtypes of iNKT-cells differ in healthy vs leukemic samples, resembling a shift in the

composition of iNKT-cells. (3) Amounts of iNKT-/NK-/CIK-cells in AML-/ALL-/CLL-MNC-samples correlate with prognosis. (4) 'Cocktail'-treated AML-blasts (resulting in DC<sub>leu</sub>) lead to a shift in T-/iNKT-/NK-/CIK-cell-counts/compositions, what correlates with improved antileukemic activity against AML-blasts-pointing to a cross-talk of these cells. Proportions of iNKT-/NK-/CIK-cells (based on 6B11/CD161/CD56/CD3-antibodies) should regularly be evaluated in AML-/ALL-/CLL-diagnosis-panels for quantitative, qualitative/prognostically relevant estimation of individual pts' antileukemic potential in detail and to learn about their role in DC/DC<sub>leu</sub>-triggered-immune-surveillance.

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**Disclosure of conflict of interest:** None.

#### P535

##### Outcome after hematopoietic stem cell transplantation for Philadelphia-positive AML: relatively favorable outcome in patients allografted in first complete response; a survey from the acute leukemia working party of the European society for blood and marrow transplantation (EBMT)

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AML with t(9;22) and BCR-ABL rearrangement (Ph-pos AML) is a very rare AML subtype, recognized as a new provisional entity in the recent WHO 2016 classification. The role of stem cell transplantation (SCT) in the era of ABL tyrosine-kinase inhibitors (TKIs) is mostly unknown. We analyzed long-term outcome in patients  $\geq 18$  years after allogeneic or autologous SCT performed between 2000–2013 in EBMT centers responding to a designated survey. Patients with blast crisis CML and Philadelphia-positive ALL were excluded. Primary end-point was OS. Secondary end-points were NRM, acute GVHD, chronic GVHD, LFS, RI, and the effect of TKI on outcome. 65 patients (median age, 48 years, range: 19–67; 31 males and 34 females) with Ph-pos AML undergoing SCT (allogeneic, 57; autologous, 8) were identified. Median WBC count at diagnosis was  $57 \times 10^9/L$  (1.2–366) and 25% had splenomegaly (data missing on 10 patients). Translocation t(9;22) was the sole abnormality in 36 patients (55.5%). The majority of the patients received one or two courses of chemotherapy before transplant and 79% attained CR after one course. The majority (70%) received a TKI (mostly imatinib, 34/43) before transplant, with a median period of exposition of 86 days (IQR 60–173), while 21 (34%) received TKI after the transplant either as maintenance ( $n=11$ ) or treatment for relapse ( $n=9$ ). At time of transplant, 56 patients were in complete response (CR1-53, including all autoSCT; CR2-3) and the remaining 9 patients were allografted in advanced phase. Among 40 patients with available information, 14 achieved a MRD negative status at transplant (ratio BCR-ABL/ABL  $< 10^4$ ). Regarding alloSCT, conditioning regimen was myeloablative (MAC) in 29/30 (96%) patients  $< 50$  years, while in patients  $> 50$  years 15 received a reduced intensity regimen (RIC) and 9 MAC. Cell source was peripheral blood stem cells in 39 and bone marrow in 18 allogeneic transplants. The donor was a HLA matched sibling (MSD) in 32 cases and unrelated (UD) in 25, amongst whom 18 were 10/10 and 7 were 9/10 HLA matched, respectively. In the 8 patients undergoing autologous SCT the majority received busulfan-based conditioning ( $n=6$ ) and peripheral stem cells ( $n=7$ ). Median follow-up was 6.3 years (0.7–14.2). Out of 26 patients MRD positive at transplantation, 16 reached MRD negativity ( $< 10^4$ ) (61.5%) after the transplantation. In patients undergoing alloSCT, NRM, RI, LFS, OS, and GRFS at 5 years was 18.1% [95% CI: 9.2–29.4], 37% [24.6–49.6], 44.2% [31.1–57.3], 53.8% [40.4–67.3], and 32.1% [19.9–44.4], respectively. As for GvHD, the cumulative incidence of aGVHD grade II-IV was 16.4% [8–27.4], cGVHD 24.9% [14.4–36.9], and extensive cGVHD 21.4% [11.7–33], respectively. Among the 44 patients receiving alloSCT in CR1, 5-yr NRM, RI, LFS, OS, and GRFS was 15.9%,

36.4%, 46.5%, 59.4%, and 34.9%, respectively. By the univariate analysis, age (< 50 vs ≥ 50) was associated with RI (5-yr: 22.7 vs 50%), LFS (5-yr: 61.9 vs 31.8%), and GRFS (5-yr: 52.4 vs 18.2%), whereas MRD-negative status before alloSCT was associated with an improved GRFS (38.9 vs 16.7%). In the 8 patients autografted, RI, NRM, LFS and OS at 5-year was 50% (12.5–79.4), 0%, 50% (15.4–84.6), and 62.5% (29–96), respectively. Outcome of patients with Ph-pos AML who received alloSCT in CR in recent years was relatively favorable, especially among younger patients, probably reflecting the beneficial effect of TKI.

**Disclosure of conflict of interest:** None.

#### **P536**

#### **Outcome of allografting for AML-CR2 is equivalent across the BSBMT and EBMT and is associated with encouraging OS and DFS across all age groups**

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Relapsed AML has a very poor prognosis with a high mortality, even if a second CR2 is achieved. The only curative treatment is with an allogeneic HSCT but allografts for AML in CR2 are considered to have a worse outcome compared to those performed in CR1, especially in older patients for whom this therapy may not be considered. The BSBMT undertook a bench-marking study analysing the outcomes for all patients allografted for AML-CR2 from 2006 to 2011. The UK outcomes from 534 paediatric and adult patients were compared to 3070 non-UK patients transplanted for the same indication reported to the EBMT during the same period. Allogeneic transplants for AML-CR2 represent an important part of any allograft program and numbers referred for allograft were stable between 2006–2011 in both programs. The median age of patients was 45.8 yrs and 43.3 years in the BSBMT and EBMT cohorts respectively, with 14% and 15% of patients aged < 18 years and 19% and 16% aged > 60 years in the 2 groups. The length of first remission was missing in many of the EBMT registrations so time from diagnosis to transplant was used as a surrogate for this and was similar in both cohorts (18 m and 19 m respectively). Similarly the presence of comorbidities was poorly reported in both databases but was similar. The BSBMT cohort included fewer patients undergoing RIC conditioning protocols (55% vs 66%), fewer sibling transplants (25% vs 38%) and more PBSC allografts (79% vs 20%). Transplant related morbidity and mortality were similar across the two cohorts (BSBMT v EBMT) with rates of severe acute GVHD (grade III and IV) 6% v 10%, limited chronic GVHD 35% v 28%, relapse at 1 year 22% v 20% and death in CR at 1 year 20% v 18%. Chronic GVHD (both limited and extensive) appeared more common in the BSBMT cohort (50% v 36%) although reporting of cGVHD was more comprehensive and complete within the BSBMT registry and may be under-reported in the EBMT registry. Outcomes were excellent in both cohorts with outstanding rates of leukaemia free survival in this high risk cohort at day 100 (BSBMT v EBMT) 86% v 83%, at 1 year 63% v 67%, at 3 years 49% v 53% and at 5 years 41% v 43%. Although OS and LFS was significantly shorter in patients aged > 60 years, at 37% and 30% the results in this high risk age group are acceptable and warrant its continued use. Multivariate analysis of the combined cohorts showed that age at transplant (< 18 yrs/18–60/> 60 yrs), time from diagnosis to transplant and the presence of aGVHD were important factors affecting DFS. Risk factors for relapse included the type of conditioning used, presence of aGVHD and time from diagnosis to transplant, whereas those for TRM included age, aGVHD, source of stem cells and time to transplant. There was no significant difference in outcomes between the BSBMT and EBMT for this indication. Outcomes for patients allografted for AML-CR2 are excellent and appear superior to those reported for patients not undergoing an allograft in both the BSBMT and EBMT cohorts. The OS and DFS observed are comparable to

those reported for allografts in AML-CR1 and, although this study has not considered outcomes for patients who did not achieve a 2<sup>nd</sup> CR, it nevertheless supports the practice of risk stratification of AML patients such that only high risk patients are offered an allograft in CR1 with the remainder being offered an allograft as salvage after relapse.

**Disclosure of conflict of interest:** None.

#### **P537**

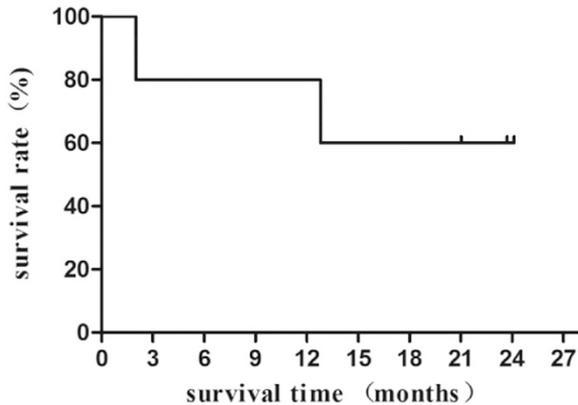
#### **Outcome of HTLV-1 associated adult T cell leukemia/lymphoma (ATL) w/o allo-HSCT with kinetic monitoring of proviral DNA load in an endemic area in the southeast of China: a consecutive study over 20 years**

J Hu, T Yang<sup>1</sup>, Z Wu<sup>1</sup>, X Zheng<sup>1</sup>, T Liu<sup>1</sup>, J Ren<sup>1</sup> and Z Chen<sup>1</sup>  
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Outcome of HTLV-1 associated adult T cell leukemia/lymphoma (ATLL) w/o allo-HSCT with kinetic monitoring of proviral DNA load in an endemic area in the southeast of China: a consecutive study over 20 years Jianda Hu\*, Ting Yang, ZhengJun Wu, Xiaoyun Zheng, Tingbo Liu, Jinghua Ren, Zhizhe Chen Background: Adult T-cell leukemia (ATL) caused by human T-cell lymphotropic virus type I (HTLV-1) infection is an aggressive malignancy with poor prognosis and the efficacy of chemotherapy is limited. To evaluate the efficacy and safety of intensive chemotherapy followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT) for ATL. A total of 21 evaluable ATL patients treated at our center from 1995 to 2015 are retrospectively analyzed. The HTLV-I proviral DNA load in peripheral blood mononuclear cells using PCR assay was developed, in comparison with HTLV-1 Tax protein expression measured by western-blot, to confirm the diagnoses and monitor the disease control. 13 patients were male and 8 patients were female. Median age was 50.8 (range 28–66) years. All obtained patient samples were subjected to flow cytometric examination and karyotype analysis. 19 patients received chemotherapy as the induction therapy while 2 quit at the time of diagnosis, 5 with DA-EDOCH regimen while 14 with other regimens such as CHOP, VCAP and AMP. DA-EDOCH regimen is a variation of dose-adjusted EPOCH regimen with the replacement of prednisone (60 mg/m<sup>2</sup> per day) by dexamethasone (15 mg/m<sup>2</sup> per day). Before the conventional regimens Bucy followed by prophylaxis donor lymphocyte infusion, both received a course of salvage chemotherapy including fludarabine and cytarabine for 5 days registered on <http://ClinicalTrials.gov> (NCT02328950). The GVHD prophylaxis consisted of ATG, CsA and MTX. The patient characteristics, therapeutic effect and survival data were collected. All patients came from the coastal area in the south-east of China. Subtype classification of these 21 ATLL were 18 acute, 2 lymphoma and 1 chronic type. The main manifestations were characterized with cutaneous and respiratory involvement, hepatosplenomegaly, lymphadenopathy and the laboratory abnormalities as leukocytosis with ATL cells, hypercalcemia and elevated serum LDH. Typical morphological characteristic of “flower cells” were observed in 85.7% cases and most of the ATLL cells are CD4+CD8-. Chromosomal abnormalities were detected in 4 cases. All 14 patients who didn't receive DA-EDOCH regimen died of disease progress, while among 5 patients with DA-EDOCH regimen, 2 achieved CR, 2 PR and 1 died. With a median follow-up of 18.6 (10–24.1) months, 3 patients respond to DA-EDOCH are still alive. 2 patients in CR achieved successful engraftment with complete donor chimerism in one month post haplo-identical transplant. Both were received prophylaxis donor lymphocyte infusion and the immunosuppressive agents were abruptly discontinued for induction of a graft-versus-ATL (GVL) effect. They keep remain alive and disease free longer than 2 years so far without severe graft-versus-host (GVHD), and the HTLV-1 proviral DNA became undetectable after allo-HSCT. CONCLUSION: It shows great promise of DA-EDOCH regimen followed by allo-HSCT to the

long-term cure of ATL with apparent clearance of the virus. Haplo-identical transplantation can be an alternative option for the ATL patients without increasing non-relapse mortality.

[P537]



**Disclosure of conflict of interest:** None.

**P538**

**Previously published**

**P539**

**Preliminary haploidentical transplantation experience in a single center**

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In the absence of an HLA-matched related donor or a good matched unrelated donor in time, haploidentical stem cell transplantation (haplo-SCT) is an option for patients requiring an allogeneic hematopoietic stem cell transplant. Substantial progress has been made in the last two decades with a dramatic improvement in patient outcomes, with some groups reporting preliminary beneficial effects similar to the ones in HLA matched unrelated donor and cord blood transplant. Several strategies have been adopted through the years for the procedure. The two strategies used in haplo-SCT are ex vivo T-cell depletion and T-cell replete transplantation. The latter can be done with a combination of immunosuppressive drugs ( Beijing approach) or with post-transplantation high-dose cyclophosphamide (post-Cy). Due to its lower cost, feasibility and practicality, post-Cy has become the most often used platform for haplo-SCT in the majority of allogeneic transplantation units worldwide. We analyzed our experience in Haplo-SCT, since the first one in March 2104 to the last one that has just been done in October 2016. We collected all complications reported, also mortality related to treatment and to the disease. We analyzed the overall survival (OS). 9 transplants were treated, with different SCT indications, the most common being acute myeloblastic leukemia ( $n=5$ , 55%), the rest of indications are exposed in figure 1. All our patients, independent of the conditioning receive post-CY as T cell depletion measure and stem cells were collected from peripheral blood. Age at the time of transplant was 40.6 years, 77% were males, 23% females, the rest of patient characteristics are listed in Table 1. In our series the treatment related mortality (TRM) was low with only 1 patient (11%) that died before the day +100. As complications, we reported 33% of hemorrhagic cystitis, 22% of sinusoidal obstruction syndrome, 22% reports systemic inflammatory response syndrome, 55% of cytomegalovirus (CMV) reactivation. Neutrophils graft is 18.2 days ( $R=14-23$ ) and the platelets graft is 24.2 days ( $R=13-34$ ). In our series we haven't reported any case of graft

failure, one of the transplanted the patient had antiHLA antibodies, this was treated with a plasmapheresis previous the stem cell infusion, and was infused with a high number of CD34+ cells ( $8 \times 10^6/\text{kg}$ ), no graft problems, and has had no complications since then with the graft. The OS for the whole group is 22 months, with a median not reach at 36 months, with 2 patient's dead at time of analysis. Two patients had a relapse after the haplo-sct (22%), both of them received lymphocyte donor infusion, sadly, neither of them responded. The haplo-SCT procedure is been adopted by many centers for high risk hematology malignancies, mainly because the fast availability of donors, and because of the preliminary results that have been reported place it as good as the unrelated donor or cord unit transplant. Our center is getting experience in these types of transplants, and our results reflect similar outcomes as larger studies. With longer follow ups we will be able to keep the trend of good results both in procedure safety and disease efficacy. OS, toxicity and TRM are expected for these high risk malignancies. In overall, the haplo-SCT seems a reasonable technique that is reflecting in our short series, the results being reported in studies worldwide at bigger scale.

**Disclosure of conflict of interest:** None.

**P540**

**Reduced intensity conditioning (RIC) allografts overcome poor prognosis associated with complex karyotype, FLT-3 ITR and alterations of chromosome 7 in high-risk (HR) acute myeloid leukaemia (AML), yielding results comparable to other indications of allograft for AML patients**

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Complex karyotype, the presence of FLT-3 ITD and losses of genetic material in chromosome 7, are all considered high risk markers in AML. Patients bearing these abnormalities could undergo a myeloablative allogeneic transplantation in CR1 whenever this is possible, although this could significantly reduce the chances for cure in older patients due to increased transplant related mortality. RIC allografts could be performed in older patients in order to overcome the deleterious effects of these individual abnormalities but its effect still remains controversial in the high risk group. Between 2005 and 2015, a total 65 patients [41 males, 24 females, mean age 59.05 (40-73)] received a RIC allograft (49 from fully matched unrelated donors, and 16 from an identical sibling) for high risk AML in 2 transplant centres. High risk disease was classified according to their response to treatment, the presence of complex karyotype, the presence of individual cytogenetic/molecular abnormalities or a combination of these. In particular, 24 patients presented one single karyotype abnormality and 17 presented three or more. Ten patients presented alterations of chromosome 7 and 7 patients presented Flt-3 ITR. The conditioning regimes included fludarabine in all cases, together with melphalan and Campath (29 patients), Busulphan and Campath (21 patients), busulphan and thiotepa (1 patient), Melphalan (3 patients), busulphan with and without ATG (9 patients) total body irradiation (200 cGy, 2 patients). Graft versus host disease prophylaxis was Ciclosporin for patients receiving Alemtuzumab or ATG but for patients who had a T-replete allograft Ciclosporin and low dose methotrexate was the preferred prophylaxis. All but four patients were transplanted in CR1 Patients were followed-up for a mean 32.3months (range 3-150). Thirty-one (47%) patients remain alive. The causes of death (34 cases) include relapse or progression of the original disease (13), transplant-related causes (17) and unknown in 4 cases. The influence of the genetic abnormalities on survival

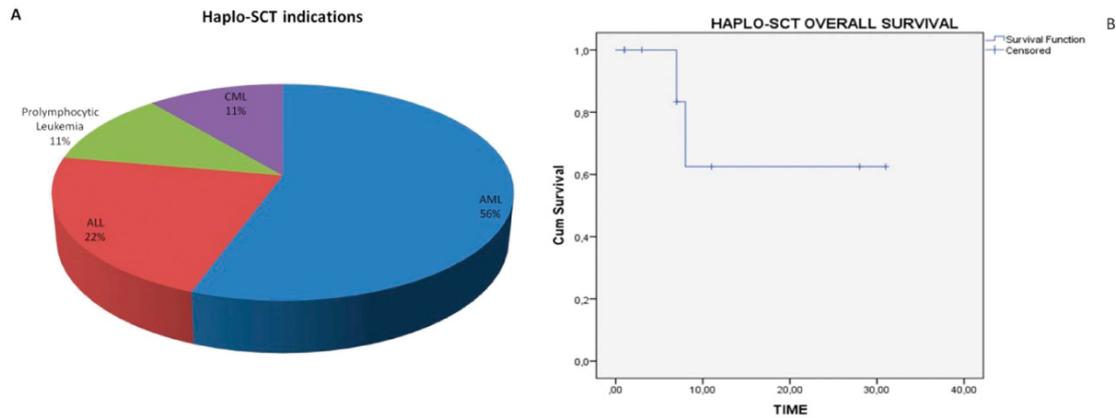


Figure 1: A: Indications for Haplo-SCT; B: Overall survival after 35 months follow up.

Patient and disease characteristics				
<b>Age</b>	40.6 years (average)	>50y= 4 (range 19-39)	<50y= 5 (range 50-66)	
<b>Gender</b>	Male 77% (n=7)		Female 23% (n=2)	
<b>Indications for SCT</b>	AML (n=5)	ALL (n=2)	T-cell prolymphocytic leukemia (n=1)	CML (n=1)
<b>Disease Risk Index</b>	Low 22%(n=2)	Intermediate 33% (n=3)	High 33% (n= 3)	Very High 11% (n=1)
<b>Donor</b>	Parents 33% (n=3)	Siblings 22% (n=2)	Brother/Sister 33% (n=3)	
<b>ABO Match</b>	Match 89% (n=8)	Minor mismatch 11%(n=1)	Major Mismatch (n=0)	
<b>Gender Match</b>	Male/Male 44% (n=4)	Female/Female 22% (n=2)	Male/Female 33% (n=3)	
<b>Conditioning protocol</b>	TBF 78% (n=7)	TBI + Etoposide 11% (n=1)	Fludarabine +TBI 11% (n=1)	
<b>Conditioning type</b>	Myeloablative protocol 33%(n=3)		Reduced Intensity Conditioning 67%(n=7)	
<b>Product infused</b>	CD34+= 4.8 x10*6/kg Avg (range 3-8)		CD3+= 2.3 x 10*8/kg Avg (range 0.7-4.5)	
<b>Days for graft (&gt;500 neutrophils)</b>	18.2 days (range 14-23)			
<b>Days for platelets graft (&gt; 20.000)</b>	24.2 days (range 13-34)			
<b>Hemorrhagic Cystitis</b>	33% (n=3)			
<b>Sinusoidal obstruction syndrome</b>	22% (n=2)			
<b>GVHD</b>	Acute (any Grade) 56% (n=5)	Acute (>Grade III) 11% (n=1)	Chronic (any Grade) 33% (n=3)	Chronic (>grade III) (n=0)
<b>CMV reactivation</b>	55% (n=5)			
<b>CMV match donor/recipient</b>	CMV+/CMV+ 77% (n=7)	CMV+/CMV- (n=0)	CMV-/CMV- (n=0)	CMV-/CMV+ 22% (n=2)

\*AML, acute myeloblastic leukemia; ALL acute lymphoblastic leukemia; CML, chronic myeloid leukemia; TBF, tiothepa, busulfan, fludarabine; TBI, total body irradiation; BUCY, busulfan, cyclofosfamide; CMV, citomegalovirus.

Table 1: Patients characteristics

was analysed, showing there were no significant differences between patients with normal karyotype, single chromosomal abnormalities and two or more abnormalities. Likewise, the Kaplan-Meier survival analysis of patients bearing Flt-3 ITD was not significantly different to the rest of the cohort ( $P=0.4$ ; 3/7 died, with only one case being related to relapse). Patients bearing chromosome 7 abnormalities (with or without other chromosomal aberrations) had a comparable, not significantly ( $P=0.6$ ) different survival to the rest of the cohort: 6/10 patients bearing this abnormality died although, most interestingly, none of these deaths were related to relapse. Our results indicate RIC allografts can provide an adequate consolidating effect in HR AML with complex karyotype, alteration of chromosome 7 or Flt-3 ITR, yielding clinical results that are comparable to those obtained in patients with AML allografted for other indications. This is particularly important as these alterations are more frequent in patients whose age prevents them from having myeloablative grafts.

**Disclosure of conflict of interest:** None.

#### P541

##### **Reduced relapse risk in patients with AML after HCMV-replication post transplantation-final results of a prospective registration trial (DRKS00004300)**

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Early detection of inapparent replicative human cytomegalovirus (HCMV) infection together with its preemptive antiviral treatment has led to a marked reduction of life-threatening HCMV disease after allogeneic hematopoietic stem cell transplantation (alloSCT). We first reported in a retrospectively performed study that HCMV reactivation is associated with a reduced risk for relapse in patients with AML after transplant. Now, we evaluate the impact of early HCMV replication on the risk for leukemic relapse in patients with AML after T cell depleted transplantation in a prospectively performed observational study (Registration Trial DRKS00004300). Between January 2012 and March 2015 we enrolled 251 patients with AML in this trial who were consecutively transplanted at the University Hospital of Essen. 239 patients received a myeloablative regimen (TBI based conditioning  $n=83$ , chemotherapy based conditioning  $n=156$ ) and 12 patients a RIC ( $n=12$  chemotherapy based regimen). Patients were transplanted in 1.CR ( $n=123$ ), 2.CR ( $n=51$ ) or more progressive disease stages ( $n=77$ ) from HLA-identical sibling donors ( $n=60$ ) or HLA-identical unrelated donors (URD) ( $n=126$ ) or mismatched unrelated donors ( $n=65$ ). Patients who received a second transplant were excluded from the study. The median age of patients was 54 years (range 18–72) and that of the donors 35 years (range 14–69). GVHD prophylaxis was performed with MTX and CSA, or CSA and MMF with ( $n=182$ ) or without ATG ( $n=69$ ) (30–60 mg total dose). The incidence of acute GVHD grade 2–4 was statistically not different in both groups (46% versus 40%). HCMV-reactivation (HCMV-R) detected by PCR occurred between 23 and 87 days (median 45 days) after allo SCT. Only patients surviving day 60 after transplant were included in the study for estimation of relapse incidence (CIR) or overall survival (OS). HCMV status of recipients (R) or donors (D) were in 29% R-/D-, 12% R-/D+, 28% D+/R- and 31% R+/D+. Patients with a documented HCMV-R had a CIR at 4-year after transplant of only 30% as compared to 47% in patients without a HCMV-R ( $P=0.016$ ). Estimates for 4-year OS were in favor for patients with HCMV-R (61% for patients with HCMV-R versus only 48% for patients without HCMV-R), but this did not achieve statistical significance. Non-relapse mortality was greater in patients with HCMV reactivation (23% versus 12%, NS) A substantial and independent reduction of relapse risk associated with early HCMV replication was confirmed by multivariate analysis including competitive factors as unfavorable cytogenetics according to ELN, advanced disease stages

of AML, HLA-identical donor versus mismatched donor, sibling versus unrelated donor, presence of acute GVHD grades II–IV, chronic GVHD, and HCMV-R [(hazard ratio: 0.61, 95% CI: 0.38–0.98,  $P<0.042$ ) for occurrence of HCMV-R]. The final result of this first prospective performed study confirms an independent advantageous effect of early HCMV replication on the leukemic relapse risk in patients with AML after transplant.

**Disclosure of conflict of interest:** None.

#### P542

##### **Results of hematopoietic stem cells transplantation with TCRαβ+/CD19+ depletion from matched unrelated and haploidentical donors in pediatric acute myeloblastic leukemia patients in complete remission**

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Graft-versus-host disease (GvHD) and GvHD-associated morbidity and mortality are major obstacles to the success of transplantation from unrelated and haploidentical (haplo) donors. Negative depletion of TCRαβ(+)/CD19+ is a technology of graft manipulation with a potential to improve GvHD control and immune reconstitution. A total of 65 pediatric patients (pts) with AML (21 f/44 m, median age 7 years, range 0.4–23) underwent allogeneic HSCT between May 2012 and July 2016. Thirty pts received haplo graft, 35 a graft from MUD. Disease status at HSCT was CR1 in 52 pts, CR ≥ 2 in 13 pts. For all pts it was first HSCT. TCRαβ+/CD19+ depletion of HSCT with CliniMACS technology was implemented in all cases. All patients received Treo-based conditioning regimen. GvHD prophylaxis regimens: in 2012–2013 ( $n=25$ ): hATG 50 mg/kg and post-HSCT Tacro/MTX ( $n=25$ ) and in 2014–2016 ( $n=40$ ): ATG (rabbit) 5 mg/kg, rituximab 200 mg/m<sup>2</sup> and post-HSCT bortezomib ( $n=39$ ) or Tacro/MTX ( $n=1$ ). The median dose of CD34+ cells in the graft was  $9 \times 10^6$ /kg (range 4.2–15), TCRαβ– $19 \times 10^3$ /kg (range 1–137). Median time of FU for survivors was 2.18 years (0.3–4.4). Primary engraftment of WBC and platelets was achieved in 64 pts, one patient (pt) died at day +3 after HSCT, and one had a secondary graft failure. A median time to WBC engraftment was 13 days (9–27), to platelets - 14 days (9–32). Cumulative incidence (CI) of acute GvHD (aGvHD) grade ≥ II was 25% (95% CI:16–38): from haplo–23%(12–45), from MUD 26% (15–45),  $P=0.7$ . CI of aGvHD > III 8%(95% CI:3–18): haplo vs MUD - 7% (95% CI:2–25) vs 9%(95% CI:3–25), respectively,  $P=0.7$ . CI of chronic GvHD (cGvHD)–16% (95% CI:9–28). CI of aGvHD was significantly lower in a group with regimen 2 (2014–2016) of GvHD prophylaxis: 10% (95% CI:4–25) vs 32%(95% CI:18–57),  $P<0.0001$ . Regimen 2 was also effective in prevention of cGvHD: CI at 2 year after HSCT was 10% vs 24%,  $P=0.14$ . CI of TRM was 12%(95% CI:6–24): haplo–7%(2–25), MUD - 14% (6–32). Early mortality (before +100-day) was relatively low ( $n=3$ ): 1 pt died from bacterial sepsis; two pts died due to AdV infection. Thirteen pts died after 100 days: 9 pts relapsed and died due to complications of second HSCT; bacterial sepsis in 1 pt and viral infection (AdV and CMV) in 3 pts (2 with extensive chronic GvHD). CI of relapse was 24% (95% CI:15–38) at 2 year: from haplo– 12% (95% CI:4–36), from MUD–32% (95% CI:20–52),  $P=0.086$ . Event-free survival (EFS) at 2 years was 64% (95% CI: 52–77): haplo - 81%(95% CI: 66–97), MUD - 54% (95% CI:37–70),  $P=0.06$ . OS was 70% (95% CI: 58–82) at 2 years: haplo–77% (95% CI: 57–77), MUD - 65% (95% CI: 48–81),  $P=0.3$ . Relapse-GvHD-free survival at 2 years was different among recipients of haplo and MUD HSCT: 51%(95% CI: 28–75) vs 32% (95% CI: 16–42),  $P=0.3$ . We confirm that the depletion of TCRαβ+/CD19+ depletion from the graft ensures high engraftment rate and acceptable transplant-related mortality in pediatric AML pts. There is a trend towards better EFS for haploidentical transplantation. GvHD prophylaxis including rATG/Rituximab/Bortezomib improves GvHD control

in recipients of TCR $\alpha\beta$ +CD19+depleted grafts in comparison to hATG/Tacro/MTX apparently without loss of anti-leukemic activity.

**Disclosure of conflict of interest:** None.

#### P543

### Results of T-cell depleted haploidentical stem cell transplantation in adults with acute leukemia improve with time: a study from the acute leukemia working party of the European society of blood and marrow transplantation (EBMT)

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T cell-depleted (TCD) transplants from haploidentical donors are increasingly used in the absence of a HLA full-matched donor for patients (pts) with high risk acute leukemia (AL). Progress has been made in optimization of conditioning regimens and post-transplant cellular therapy to potentiate the graft-versus-leukemia effect with no graft-versus-host disease (GvHD). However, relapse incidence (RI) and non relapse mortality (NRM) remain the main obstacles for pts outcomes. We report 308 adults with de novo AL, given a TCD haplo from 2005 to 2015. To analyze the effect of transplant period on TCD haplo outcomes, pts were analyzed in two separate groups: 2005–2011 ( $n=191$ ) and 2012–2015 ( $n=117$ ). TCD haplo were performed in 66 EBMT centers. Median follow-up was 11 months with no difference according to transplant periods. Median age was different between groups, being 41 and 46 years respectively ( $P=0.044$ ). The majority of pts had acute myeloid leukemia (AML) (75% vs 69%,  $P=0.261$ ) and disease status at haplo was first complete remission (CR1) in 55% and 64% of pts respectively ( $P=0.115$ ). Pts transplanted before 2012 had more frequently a Karnofsky performance status  $< 90\%$  (19% vs 10%,  $P=0.041$ ). Conditioning was myeloablative in 76% and 77% of TCD haplo before and after 2012 ( $P=0.935$ ), mainly based on Fludarabine(Flu)-TBI, Flu-Melphalan-Thiotepa or Cyclophosphamide-TBI. As for RIC it was Flu-Melphalan-Thiotepa, Flu-TBI or Cyclophosphamide-TBI. The cumulative incidence (CI) of neutrophil engraftment, grade II-IV acute GVHD and chronic GVHD were not different according to transplant period, being 93% and 90%,  $P=0.302$ ; 20% and 22%,  $P=0.667$ ; 19% and 11%,  $P=0.119$ , respectively. In the whole population 2-year RI and NRM were 20% and 48%, with no difference before and after 2012 (21% vs 19%,  $P=0.722$ ; 54% vs 38%,  $P=0.109$ , respectively). RI was 20% before 2012 versus 15% after 2012. The main cause of NRM was infection, with no difference over time (46% vs 50%,  $P=0.316$ ). In multivariate analysis, disease status was the only factor associated with RI (HR 2.05, 95% CI 1.09–3.84,  $P=0.025$ ). TCD haplo after 2012 (HR 0.57, 95% CI 0.38–0.86,  $P=0.008$ ), younger age (HR 0.82, 95% CI 0.63–0.98,  $P=0.023$ ) and RIC (HR 0.53, 95% CI 0.32–0.88,  $P=0.014$ ) were independently associated with lower NRM. 2-year OS was 36% with a marked improvement for TCD haplo performed after

2012 (47% vs 29%,  $P=0.024$ ), while LFS and GRFS were 31% and 24%, respectively. According to disease status, 2-year LFS, OS and GRFS were higher for pts transplanted in CR1 (38% vs 22%,  $P<0.001$ ; 41% vs 29%,  $P=0.006$ ; 30% vs 17%, respectively  $P=0.004$ ). In multivariate analysis, TCD transplant after 2012 (HR 0.58, 95% CI 0.41–0.84,  $P=0.003$ ) and RIC regimen (HR 0.64, 95% CI 0.42–0.97,  $P=0.034$ ) were associated with better LFS. Similarly, TCD haplo performed after 2012 (HR 0.54, 95% CI 0.38–0.79,  $P=0.003$  and HR 0.56, 95% CI 0.39–0.79,  $P=0.001$ ) and RIC (HR 0.54, 95% CI 0.35–0.83,  $P=0.005$  and HR 0.59, 95% CI 0.38–0.90,  $P=0.016$ , respectively) were independently associated with better OS and GRFS. Outcomes of TCD haploidentical transplant improve over time. In our analysis NRM, LFS, OS and GRFS have progressively improved in transplants performed after 2012. One possible explanation is gaining experience by centers, new conditioning regimens, better supportive care and new post-transplant cellular therapies that results in reducing TRM and facilitating immune recovery.

**Disclosure of conflict of interest:** None.

#### P544

### Sequential conditioning and haploidentical transplantation produces long-term survival in high-risk AML and is equally effective to HLA-matched SCT—Results of a matched-pair analysis

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HLA-haploidentical allogeneic stem cell transplantation (haploSCT), followed by post-transplant cyclophosphamide (ptCY) for immunosuppression is effective in Acute Myeloid Leukemia (AML) in complete remission (CR). For high-risk and advanced AML, less promising results were reported. Sequential conditioning regimens, comprising cytoreductive chemotherapy shortly before reduced intensity conditioning (RIC, e.g. FLAMSA-RIC, Schmid, Blood 2006), have been successfully used for matched family and unrelated SCT in high-risk AML, and therefore have made their way into conditioning protocols for haploSCT (Tischer, Ann Haematol, 2013). To further establish the role of haploSCT in high-risk AML, we performed a retrospective matched-pair comparison of HLA-matched SCT vs haploSCT/ptCY in two German centers. High-risk AML was defined by any of the following criteria: Refractory or relapsed AML, secondary AML, or genetic aberrations classified as intermediate-high or adverse according to the ELN classification. All consecutive adults, who fulfilled  $\geq 1$  of these criteria before either HLA-matched or haploSCT/ptCY were included ( $n=200$ ). Recipients of haploSCT were pair-matched with patients receiving a matched donor SCT. Matching variables were (1) stage at SCT, (2) genetic subgroups accordingly to ELN, and (3) age ( $\pm 5y$ ). 39 patients (pts) undergoing haploSCT/ptCY could be successfully pair-matched ( $P=1.0$  for stage and genetic subgroup, 0.9 for age) with 39 recipients of matched SCT (12 family, 27 unrelated SCT). Within the entire cohort, median patient age was 57y (24–70). At start of conditioning, 20% of patients were in CR, 18% had refractory, 52% had relapsed, and 10% had untreated disease. Genetics were favorable (16%), intermediate I (51%), intermediate II (12%) and unfavorable (21%). HLA-matched SCT was uniformly performed following FLAMSA-RIC. 34 recipients of haploSCT/ptCY (87%) received cytoreductive chemotherapy with FLAMSA ( $n=16$ ) or Clofarabine ( $n=18$ ) before RIC was started. Median follow-up among survivors was 33 months. Overall CR rate at d+30 was 95%, 4 patients suffered from refractory disease or early death, ( $n=2$  each). Overall-Survival (OS) for the entire cohort was 74%/53.9% at

1y/3y from SCT. The corresponding 1y/3y leukemia-free survival (LFS) was 63.5%/46.6%. Median time to engraftment was 18.0 and 17.5 days after matched and haploSCT, respectively ( $P=0.8$ ). With respect to outcome, no difference was observed between the two groups: OS at 1y/3y was 78.5%/54.5% after matched SCT, and 61.5%/55.2% after haploSCT/ptCY ( $P=0.71$ , Figure 1). LFS at 1y/3y was 76.2%/42.6% within the HLA-matched group and 56.4%/50.8% within the haploidentical group ( $P=0.99$ ). Recipients of haploSCT showed a higher incidence of aGVHD  $\geq$  II° (46% vs 18%,  $P=0.014$ ), as well as a trend towards increased 1y-NRM (18% vs 5%,  $P=0.08$ ), whereas 1y-relapse rates were comparable (23% after haploSCT/ptCY vs 26% after matched SCT,  $P=0.5$ ). Relapse was the most frequent cause of death in both cohorts, main causes of NRM were GVHD and infections (no difference between the two groups). Allogeneic SCT following sequential conditioning can achieve excellent results in high-risk AML. In our study, results after haploidentical transplantation were comparable to those obtained after HLA-matched SCT. Hence, haploSCT/ptCY following sequential conditioning can be considered as a reasonable option for patients with high-risk AML.

[P544]

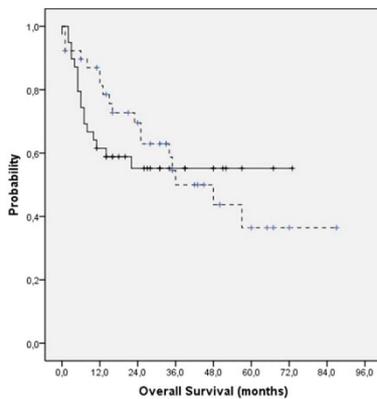


Figure 1: Overall survival after allogeneic SCT for high-risk AML from haploidentical (solid line) or HLA-matched (dashed line) donors ( $p=0.71$ )

**Disclosure of conflict of interest:** None.

**P545**

**Sequential fludarabine, Ara-C, etoposide (FLAV) followed by Fludarabine/Busulfan reduced toxicity conditioning is safe and effective salvage for adult patients with refractory acute myeloid leukemia and high risk myelodysplasia**

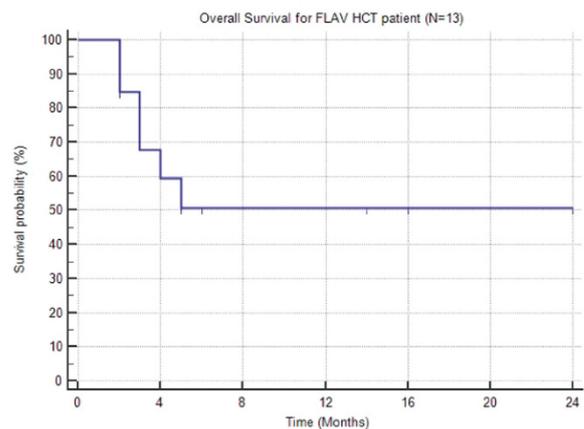
A Al-Otaibi<sup>1</sup>, S Ahmed, G El-Gohary<sup>2</sup>, N Al-Hashim<sup>3</sup>, W Chebbo<sup>2</sup>, F Alenazi<sup>3</sup>, A Hakim<sup>3</sup>, M Al-Humaid<sup>3</sup>, H Alsaadi<sup>3</sup>, M Alsermani<sup>2</sup>, F Anjum<sup>2</sup>, A Mannan<sup>2</sup>, M Hassanein<sup>2</sup>, A Balbaid<sup>3</sup>, H Almahayni<sup>2</sup>, T Hussain<sup>2</sup>, A Hanbali<sup>2</sup>, M Shaheen<sup>2</sup>, F Alfraih<sup>2</sup>, R Elfakih<sup>2</sup>, R Younis<sup>4</sup>, S Hashmi<sup>2</sup> and S Ahmed<sup>2</sup>

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A significant proportion of patients with acute myeloid leukemia (AML) will either be refractory to initial chemotherapy or will suffer refractory relapse. The role of allogeneic transplantation (HCT) in active disease is contentious. There is a growing body of literature that sequential chemotherapy, pioneered by the German FLAMSA regimen, followed by RIC HCT is a safe and efficacious modality in these patients, and there have been numerous modifications of this regimen, especially as amsacrine is not widely available. Fludarabine,

cytarabine and etoposide (VP16) (FLAV) have been reported as an effective salvage regimen. Here we report on single center outcomes of a variation of the FLAMSA regimen, substituting amsacrine for etoposide with mainly myeloablative conditioning. Patients were consented for a clinical protocol if they had AML that was refractory to 2 cycles of chemotherapy, or 1 cycle and considered to be at risk of complications of a second cycle, and if they had a matched related donor. Patients with myelodysplasia received FLAV if they had high or very high risk cytogenetics. Cytoreductive chemotherapy consisted of fludarabine 30 mg/m<sup>2</sup>/day × 4 days, Cytarabine 2g/m<sup>2</sup>/day × 4 days, etoposide 100 mg/m<sup>2</sup>/day × 3 days, commenced simultaneously. After 3 days of rest, conditioning chemotherapy consisted of fludarabine 30 mg/m<sup>2</sup> × 2 days and IV busulfan 0.8 mg/m<sup>2</sup> q 6 hours; the number of busulfan doses varied between 8–12, depending on patient comorbidity. Ten patients (76%) received myeloablative doses of busulfan. Patient received 2 doses of ATG at 2.5 mg/m<sup>2</sup>/day on day -3 and -2. Patients received G-CSF mobilized peripheral blood hematopoietic cells. Post-transplant GVHD prophylaxis was CsA and MMF. CsA was tapered from day+60 and stopped at day +90 in the absence of GVHD. MMF was discontinued between day +30 and day +40. Donor lymphocyte infusions were collected for planned prophylactic DLI. Thirteen patients received a FLAV-SCT between March 2014 and October 2016. The median age was 39(15–57); male:female ratio was(7: 6). 10 patients (77%) had AML and 3 (23%) pts had MDS. All patients had detectable disease prior to FLAV. The median time for plt engraftment was 19 days (9–50). The median time for ANC engraftment was 15 days (10–26). Cytogenetic CR rate on a day 28–30 bone marrow was 46%, and morphological CR was 60%. 3 patients (23%) developed veno-occlusive disease. Acute GVHD grade II-IV occurred in 4 pts (30%). 3 (23%) patients developed chronic GVHD. Death was due to disease relapse in 5 (38%) and NRM in 1(7.7%) patients, resulting from H1N1 pneumonia. 5 patients (38%) received DLI for post transplant relapse, and one of these is in molecular remission. At a median follow up of 4.8 months post transplant (1.6–29m), 1 year DFS was 23%. The 1 year and 2 year OS was 51% (±14%) (Figure 1). Our experience, consistent with published data, demonstrates that for patients with active AML refractory to chemotherapy, transplantation is an effective modality of disease control and may be the only curative therapy in a significant proportion. Etoposide appears to be a suitable alternative to amsacrine. Our patients tolerated busulfan at myeloablative doses, and this may be required for adequate disease control. Our report is limited by small numbers and relatively short follow-up, but is encouraging enough for us to continue offering FLAV HCT for these high-risk patients.

[P545]



**Disclosure of conflict of interest:** None.

**P546****Sequential high-dose chemotherapy reinduction followed by haploidentical transplantation in acute leukemias**

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Outcomes after T-cell replete haploidentical stem cell transplantation (Haplo HSCT) have been encouraging and Haplo HSCT has become an alternative option for patients without a HLA-identical related or unrelated donor.<sup>1,2</sup> As previously published, the sequential use of intensive chemotherapy and allogeneic transplantation represents a possible approach to the treatment of high-risk Acute Myeloid Leukemia (AML).<sup>3,4</sup> Between 2010 and 2015, 19 acute leukemia (AL) patients received Sequential Therapy (S.T.) consisting of high-dose chemotherapy reinduction and Haplo HSCT during the chemotherapy-induced neutropenia. Median age at transplant was 50 years (range: 21–62); median number of previous therapy lines was 2 (range: 1–3) and 5/19 (26%) patients had received a previous allogeneic HSCT. Twelve and 5 out of 19 patients had *de-novo* AML and secondary AML, respectively; furthermore two patients presented Blastic Crises of Chronic Myeloid Leukemia. All patients had active disease at the time of S.T. and median marrow blast count before reinduction was 25% (range: 6–88%). Hematopoietic Cell Transplantation Comorbidity Index was  $\geq 3$  in 12/19 patients (63%). All patients received high-dose Cytarabine ( $\geq 1$  g/sqm) containing regimens as reinduction therapy. The conditioning regimen was started after a median of 9 days from the end of reinduction (range: 4–15). Sixty-eight percent of patients (13/19) were conditioned with a myeloablative regimen (Thiotepa tot. 10 mg/kg, Busulfan tot. 9.6 mg/kg, Fludarabine tot. 150 mg/sqm), while 6/19 (32%) patients received a non-myeloablative conditioning. Bone marrow was used as stem cell source in 18/19 (94%) patients. Graft-versus-host disease (GVHD) prophylaxis consisted of post-transplant Cyclophosphamide with calcineurine inhibitors and mycophenolic acid. All patients engrafted but one, who was rescued with a second Haplo-HSCT with peripheral blood stem cells from the same donor. Median day of neutrophil recovery was day +18 (range: 14–24). Median follow-up of survivors was 4.2 years (range: 1.6–5.4); 1-year Overall and Event-Free Survivals were 37% and 32%, while 1-year relapse incidence and non relapse mortality were 42% and 26%, respectively. Overall cumulative incidences of acute and chronic GVHD were 39% and 38% at day +100 and +400. Among patients who developed GVHD, 2 grade III-IV acute GVHD and 2 moderate-severe chronic GVHD were observed. At 4.2 years post Haplo-HSCT, 20% of patients are alive and disease free. In our cohort of heavily pre-treated and high-risk patients, S.T. with a myeloablative conditioning was safely used to reduce leukemic burden pre-transplant and enhance Graft-versus-leukemia effects. Only the prompt availability of a haploidentical donor allowed to implement this treatment modality. Though small the patient cohort, our findings suggest that transplant-related toxicity was acceptable and early relapse was the major treatment-failure. However, long-term survival and disease-free rates of 20% in these very poor prognosis patients are highly encouraging.

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**Disclosure of conflict of interest:** None.

**P547**

**Omitted**

**P548**

**Sustained molecular remission in an elderly patient with RCSD1-ABL1 positive acute lymphoblastic leukemia enabled by dasatinib and allogeneic stem cell transplantation**

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In elderly patients with acute lymphoblastic leukemia (ALL) and kinase activating lesions allogeneic stem cell transplantation (allo-SCT) is considered to be the only curative option.<sup>1</sup> However, high risk of relapse and non-relapse mortality (NRM) often withholds elderly patients with existing comorbidities from definitive therapy.<sup>2</sup> Even though, age alone as the most important eligibility criterion for allo-SCT has become less important.<sup>3</sup> A 61-year old patient with a medical history of adipositas, hypothyreosis, arterial hypertension, hypercholesterolemia and coronary artery disease with stent implantation was diagnosed with common-B-cell-ALL based on immunophenotype. Cytogenetic analysis showed two coexisting clones with an abnormal karyotype of 47,XX; t(1;9)(q22;q34), +17 [3] and 46,XX; t(1;9)(q22;q34),der(6)(6;17)(p23;q21) [2] and no evidence of *BCR-ABL1* positive disease using fluorescence in situ hybridization (FISH) technique. RT-PCR and sequencing of the fusion transcript was performed to validate the *RCSD1-ABL1* t(1;9)(q22;q34) fusion between exon 3 of *RCSD1* and exon 4 of *ABL1*. Western analysis of phosphorylated ABL1 and its downstream target CRKL was performed to investigate the *in vivo* activity of dasatinib. Clinical monitoring of minimal residual disease (MRD) levels has been performed via RT-PCR of the *RCSD1-ABL1* fusion transcript followed by nested PCR of the amplicon to detect early relapse or MRD. As positive control the plasmid PCR2.1-TOPO\_RCSD1-ABL1(626bp) was synthesized encoding *RCSD1-ABL1* amplicon with the fusion site. Our patient was enrolled on GMALL elderly (01/2003) and treated accordingly. Thereby, no sustained remission could be achieved. FLAG-Ida re-induction and study treatment with an oral PI3K/mTOR inhibitor remained futile. Cytogenetics and verification of the *RCSD1-ABL1* fusion gene prompted salvage treatment with the tyrosine kinase inhibitor (TKI) dasatinib as single agent. The *in vivo* activity of dasatinib was highlighted by a decrease of *RCSD1-ABL1* amplicon and inhibition of phosphorylated ABL1 and its downstream target CRKL was shown. Clofarabine and cyclophosphamide complemented treatment and MRD negative remission was achieved due to administration of the bi-specific T-cell engager blinatumomab. Consolidation with allo-SCT was performed. Ongoing remission has been achieved for more than 30 months now. We demonstrate that monotherapy with TKI like dasatinib is effective in refractory *RCSD1-ABL1* positive ALL. To the best of our knowledge, this is the first elderly patient with *RCSD1-ABL1* positive ALL with a sustained and ongoing complete remission. Thereby, we suggest allo-SCT after successful TKI even for elderly patients with existing comorbidities and uncommon cytogenetics.

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**Disclosure of conflict of interest:** None.

**P549**

**Sustained remission with Sorafenib treatment for extramedullary FLT3+-AML relapse after allogeneic stem cell transplantation**

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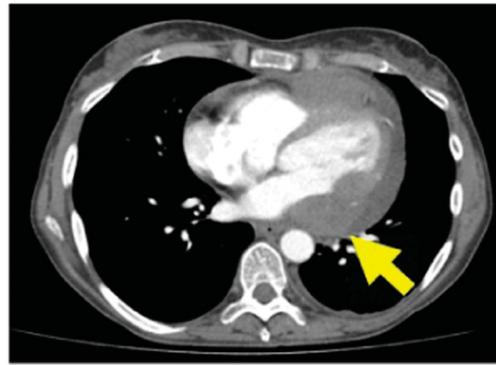
Relapse is the most important cause of failure of allogeneic hematopoietic stem cell transplantation (HSCT) for FLT3-ITD-positive acute myeloid leukemia (AML). In those cases,

treatment with FLT3 tyrosine kinase inhibitors (TKI) constitutes a promising clinical approach to induce remission without conventional chemotherapy. Forty-eight-year-old woman was diagnosed of AML secondary to myelodysplastic syndrome with NPM1 mutation and internal tandem duplications of the FLT3 gene (FLT3-ITD). After achieving Complete Remission (CR), she received a sibling allogeneic-HSCT. Four months later, AML relapsed at the medullary level, without central nervous system (CNS) involvement, treated with conventional chemotherapy and donor lymphocytes infusions (DLI). She achieved second CR and developed chronic graft-versus-host disease (cGVHD). Nine months later, she suffered the first extramedullary relapse, at the mammary, cutaneous and probably pericardial levels. There was not medullary involvement. Disappearance of the lesions at all levels was achieved with conventional chemotherapy and radiotherapy, and full donor chimerism. Eight months later, she referred atypical precordial pain irradiated to the back. Cardiac MRI was performed in which several masses were visualized in a pericardial sac up to 5 cm in diameter (Dec-15). BM was maintained in CR. In study of pericardial fluid, infiltration by leukemic FLT3 positive cells was observed. The patient was not considered candidate for further systemic chemotherapy nor radiotherapy treatment. Then, treatment with the FLT3 Sorafenib inhibitor was started, by compassionate use, at dosage of 200 mg/12 h, which maintains after one year. After

[P549]



Dec-15



Jan-16



Oct-16



first month with Sorafenib, pericardial lesions decreased considerably, ranging from 5 cm in diameter to 1.7 cm (Jan-16). In the subsequent CT controls, progressive decrease of the lesions has been observed and no new lesions have appeared in other locations. In the last CT (Oct-16) pericardial thickening is almost non-existent, without new lesions. After one year of treatment, she maintains CR at medullary and extramedullary levels Images. In our patient, treatment with Sorafenib has achieved sustained control of extramedullary disease, which had escaped the mechanisms of action of conventional chemotherapy, allotransplant and DLI. Further studies are needed to corroborate the efficacy of FLT3 inhibitors in the control of AML extramedullary disease and in the treatment of relapses after allo-HSCT.

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**Disclosure of conflict of interest:** None.

#### P550

##### TCRαβ+/CD19+ depletion in hematopoietic stem cells transplantation from matched unrelated and haploidentical donors following treosulfan or TBI-based conditioning in pediatric acute lymphoblastic leukemia patients

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Relapse, graft-versus-host disease (GvHD) and GvHD-associated mortality are major obstacles to success of transplantation from unrelated (MUD) and haploidentical (Haplo) donors in children with acute lymphoblastic leukemia (ALL). Future directions will focus on optimizing conditioning regimens and enhancing graft-versus-leukemia effect. Negative depletion of α/β(+) T cells and CD19+ B lymphocytes, which permits to maintain mature donor-derived natural killer cells and γδ (+) T cells in the graft, may improve GvHD control, immune reconstitution and prevent the relapse. A total of 71 pts with ALL (T-ALL- 27, B-ALL-44, 32 female, 39 male, median age 9.1 years (0.15–20) underwent allogeneic HSCT between May 2012 and September 2016. Forty-six pts received Haplo graft, 25 – a graft from MUD. Disease status at transplant was CR1 in 19pts, CR2 in 39 pts and CR>2 13 pts. Transplantation in CR1 was performed according to risk stratification scheme in the current institutional ALL protocol. Fifty pts received treosulfan-based myeloablative preparative regimen (71%), TBI-based regimen was used in 21 pt (29%). Two regimens of GvHD prophylaxis were used: regimen1 (n=28): ATG (horse, ATGAM) 50 mg/kg, post-grafting immunosuppression consisted of short course Tacro/MTX (n=20) before day+30 or no post-transplant prophylaxis (n=8); regimen2 (n=43): ATG (rabbit, thymoglobuline) 5 mg/kg, rituximab 200 mg/m<sup>2</sup>, bortezomib on day -5,-2 (n=29) and post-transplant bortezomib (n=26) or Tacro/Mtx (n=9), no post-transplant prophylaxis (n=8). All pts with TBI-based regimen received rabbit ATG. TCRαβ+/CD19+ depletion of HSCT with CliniMACS

technology was implemented in all cases according to manufacturer's recommendations. The median dose of CD34+ cells in transplant was 10×10<sup>6</sup>/kg (range: 3.9–18.8), TCRα/β–23×10<sup>3</sup>/kg (range: 0.2–300). Primary engraftment was achieved in 68 of 71 pts. (2 pts died before engraftment, one received second HSCT), the median time to neutrophil and platelet recovery was 13 and 14 days, respectively. Early (100 day) mortality was 7%(95% CI: 3–17), 2-year pTRM–17% (95% CI: 10–30). The three early deaths were due to bacterial sepsis (n=2) and viral infections(n=1), seven late: viral infection in 4 pts (ADV=2, ADV+CMV=1, CMV=1), bacterial sepsis in 2 pts and rhinocerebral mucormycosis in 1 pt, all late deaths were associated with cGvHD and prolonged corticosteroid therapy. CI of acute GvHD grades II–IV and III–IV was 22.5% (95% CI: 9.6–53), and 7% (95% CI: 1.3–37), respectively. CI of cGvHD was 17.6% (95% CI: 6.4–48). Regimen 2 was more effective in prevention of aGvHD II-IV: CI at 2 year after HSCT was 14% vs 35,7% in regimen 1, P=0.033 and in cGvHD 7% vs 35.7%, P=0.006. CI of relapse at 2 years was 32% (95%CI:15.7–64.5). Two-year pEFS(event = death or relapse) was 49.9% (95% CI: 37–62), 2-year pOS–54% (95% CI:41–67). In patients, who received TBI-based conditioning pEFS was 59% (95% CI: 38–81), as compared with treosulfan-based 47% (95% CI: 32–61), P=0.65. Median time of follow-up for survivors was 2.2 years (range: 0.3–4.4). We confirm that the depletion of TCR-alpha/beta and CD19 lymphocytes from the graft ensures high engraftment rate and acceptable transplant-related mortality in pediatric ALL patients. Viral infections and leukemia relapse await further improvement of control. All major outcomes were equivalent between transplantation from unrelated and Haplo donor.

**Disclosure of conflict of interest:** None.

#### P551

##### The efficacy and toxicity of blinatumomab in patients with relapsed/refractory acute lymphoblastic leukemia: A Russian multicenter experience

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The prognosis of patients (pts) with relapsed/refractory acute lymphoblastic leukemia (ALL), especially after allogeneic hematopoietic stem cell transplantation (allo-HSCT), is very poor. Therapeutic options for these pts are limited. Blinatumomab is a bispecific T-cell engager (BiTE) antibody construct with dual specificity for CD19 and CD3. BiTE therapy may help to overcome the resistance to chemotherapy (CT) with minimal toxicity, and may be a bridge to allo-HSCT. We analyzed data of 34 pts from 4 hematologic centers in Russian Federation with relapsed CD19 positive ALL, who received BiTE. The median age was 22 (range: 1–62) years, 9 (26%) pts < 18 yrs, 25 (74%) pts ≥ 18 yrs. The diagnosis was ALL B-I (EGIL) subtype in 11 pts, B-II-in 16, B-III-in 5 pts, and 1 patient had mixed phenotype leukemia (M5 (FAB) and B-ALL). Three (9%) pts had Philadelphia positive (Ph+) ALL. In 11 pts it was the first relapse of ALL, in 13–second, in 10–third. Thirty pts had isolated bone marrow relapse, 4 pts–combined relapse (bone marrow and extramedullary sites). The bone marrow blast infiltration was < 50% in 15 pts, > 50% in 19 pts. Disease relapse was revealed after CT in 19 pts (7 (37%) pts received allo-HSCT after the therapy of relapse), after allo-HSCT (3–from related, 9–from unrelated, 3–from haploidentical donors)–in 15 pts (4 pts received second allo-HSCT after the therapy). In 8 pts with posttransplant relapse donor lymphocyte infusion (DLI) was used in combination with BiTE. Every patient

received from 1 to 7 cycles (median 2) of BiTE. Complete remission (CR) was achieved in 18 (53%) pts (in 14 (41%) pts it was minimal residual negative remission): in 8 (42%) pts with ALL relapse after CT, in 10 (67%) pts—after allo-HSCT. Pts with less than 50% blasts in bone-marrow at baseline experienced substantially higher response rates compared with patients with 50% blasts or higher (67% (10/15) vs 52% (10/19)). Response rates were similar although the number of relapse—45% (5/11) in first relapse, 61% (8/13) in second relapse and 70% (7/10) in third relapse. Pts with posttransplant ALL relapse who received BiTE in combination with DLI had higher response rate than pts, who received BiTE as monotherapy: 87.5% (7/8) vs 43% (3/7), respectively. One-year OS was 50% (95% CI 23–77%). One-year DFS was 48% (95% CI 21–75%). Grade  $\geq 3$  hematological toxicity (neutropenia, thrombocytopenia) was observed in 13 (38%) pts, grade  $\geq 3$  liver toxicity—in 4 (12%) pts. Five patients (15%) developed toxic neuropathy during the therapy. Cytokine release syndrome occurred in 3 (9%) pts. One patient after allo-HSCT (but not after DLI) developed grade I aGVHD. There were no fatal treatment related toxicity. Tree (17%) pts who responded to BiTE had relapse. Eighteen (53%) pts died: 14 pts—of disease relapse/progression. The treatment of relapsed/refractory ALL with BiTE is effective and has acceptable toxicity. We demonstrated high efficacy in therapy of posttransplant ALL relapses, especially when BiTE was combined with DLI, perhaps, due to additional immunological effect of the transplant.

**Disclosure of conflict of interest:** None.

#### P552

**The mechanism of sorafenib anti-leukaemic effect seen in AML patients relapsing post alloHSCT involves the augmentation of alloreactivity which includes infiltration of the affected marrow by CD8+ cells having PD-1 receptor which presence characterize lymphocytes with anti-tumour potential**

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Multikinase inhibitor (MKI) sorafenib is clinically active in acute myeloid leukaemia (AML) patients, especially in those with *FLT3* ITD who receive alloHSCT as a part of their primary treatment. To throw some light on the mechanism of this anti-leukemic post-transplant sorafenib effect we studied the fate of two patients (*FLT3* ITD-positive, NPM1-positive) who relapsed 56 (53-year-old male) and 256 (50-year-old female) days post-transplant and their salvage treatment included sorafenib. The multikinase inhibitor (400 mg twice daily) was given either together with FLAG or DA 2+5. The response was prompt. The patients became, after completion of the chemotherapy, leukaemia free. Both patients continued the sorafenib (200 mg twice daily) treatment together with the AML standard maintenance chemotherapy (female case) or without any chemotherapy (male patient, substantial comorbidity and liver toxicity). (1). The patients responded well to the therapy and were free of leukaemia for 16 and 32+ months after initiation of the MKI treatment (*Flt3* ITD negative, 100% chimerism documented in the blood and in the marrow). (2). In both patients, 3 and 25 months on Sorafenib, skin lesions appeared either in the context of cGVHD, which progressed to a life-threatening level in a male patient or as a photodermatitis-like cheek eruption. Histopathology revealed the presence of severe CD3+ cells infiltration in affected tissues in both patients. (3). CD8 positive lymphocytes colonized the marrow of both patients. These marrows infiltrating cells co-expressed CD279 (PD-1 receptor) in proportions which were higher than those seen in the blood (14.72%  $\pm$  1.45% vs. 3.63%  $\pm$  1.21%,  $P=0.002$ ). A similar observation was made for CD8+CD69+ cells (37.26%  $\pm$  3.50%

vs. 1.58  $\pm$  0.43%,  $P < 0.002$ ). 5. Transcriptome analysis of the marrow cells, which addressed the genes involved in lymphocyte activation, revealed the presence of pro-inflammatory profile which included a higher expression of *TLR9* and *IL-12*. (1) Sorafenib given with or without moderate chemotherapy was effective in two patients in maintaining the anti-leukaemic effect of salvage chemotherapy. (2) This was associated with the presence of alloreactivity (affected tissues infiltration with CD3+ cells) clinically seen as a severe fatal cGVHD aggravated by sorafenib treatment associated unwanted effects in one cases and with rather mild skin lesions appearing later during the treatment. (3) The mechanism of anti-leukaemic effect of Sorafenib in AML patients relapsing post alloHSCT is likely due to the infiltration of the affected marrow by lymphocytes having CD279 (PD-1) what characterizes tumour infiltrating lymphocytes with anti-cancer cells killing potential.

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#### P553

**The outcome of elder patients with acute myeloid leukemia or high risk myelodysplastic syndrome treated with allogeneic hematopoietic stem cell transplantation**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative-intent treatment for patients with high-risk hematologic diseases, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Both the incidences of AML and MDS increase with age and patients elder than 60 years of age were traditionally excluded from HSCT because of high possibilities of therapy related morbidity/mortality. Recently, with the introduction of reduced intensity conditioning regimens and the improvement of HSCT care, more and more elder patients could undergo HSCT for consolidation or salvage purposes. However, literature regarding the treatment outcome of elder patients receiving HSCT is scarce. Patients diagnosed as AML or high risk MDS aged equal or more than 60 years were recruited consecutively at National Taiwan University Hospital. The high risk MDS was defined to include myelodysplastic syndrome with excess of blasts-1 and 2 according to the 2016 World Health Organization (WHO) criteria. The cytogenetic risk stratification was based on original Medical Research Council classification. From 2008 to 2016, a total of 51 patients were enrolled consecutively. The median age was 63.1 (range: 60–73) years and the gender distribution was even. Among them, 11 (21.6%) patients had high risk MDS, 27 (53%) had *de novo* AML, 10 (19.6%) had secondary AML, and three (5.9%) had therapy related AML. At diagnosis, four (7.8%) patients had extramedullary disease. Nine (17.6%) had unfavorable-risk cytogenetics, 12 (23.5%) had either unfavorable-risk cytogenetics or intermediate-risk cytogenetics but with *FLT3*-ITD mutations. Regarding the pre-HSCT disease status, nine patients had the first complete remission (CR), 11 had the second CR, and 31 patients were treatment naive or had refractory disease. The graft-versus-host-disease (GVHD)-free/relapse-free survival (GRFS) in which events include grade 3–4 acute GVHD, chronic GVHD with moderate severity according to CIBMTR criteria, relapse, or death of any cause. With median follow-up of 38 months (range: 0.8–85.2), the median overall survival (OS) for all patients was 13.9 months, the relapse-free survival (RFS) was 11.3 months, and the GRFS was 9.0 months. In univariate analysis for OS and RFS, high-risk MDS was a favorable prognostic factor but secondary or therapy related disease ( $P=0.013$  for OS and 0.007 for RFS, respectively), unfavorable-risk cytogenetics or intermediate-risk cytogenetics but with

FLT3-ITD mutations ( $P=0.005$  and  $0.002$ , respectively), pre-HSCT refractory disease ( $P=0.018$  and  $0.037$ , respectively), and grade 3–4 acute GvHD ( $P=0.001$  and  $0.002$ , respectively) were unfavorable prognostic factors. However, for GRFS, only unfavorable-risk cytogenetics or intermediate-risk cytogenetics but with FLT3-ITD ( $P=0.020$ ) and pre-HSCT refractory disease ( $P=0.018$ ) were unfavorable prognostic factors. In multivariate Cox proportional hazards regression analysis for OS and RFS, grade 3–4 acute GvHD was a significant unfavorable risk factor; for GRFS, pre-HSCT refractory disease status was a significant unfavorable risk factor. Our results showed that the choice of HSCT should not solely based on the age factor and pre-HSCT disease status. Incorporating cytogenetics and genetic mutation status could risk-stratify elder patients with HSCT. Further prospective trials are warranted to validate these findings.

**Disclosure of conflict of interest:** None.

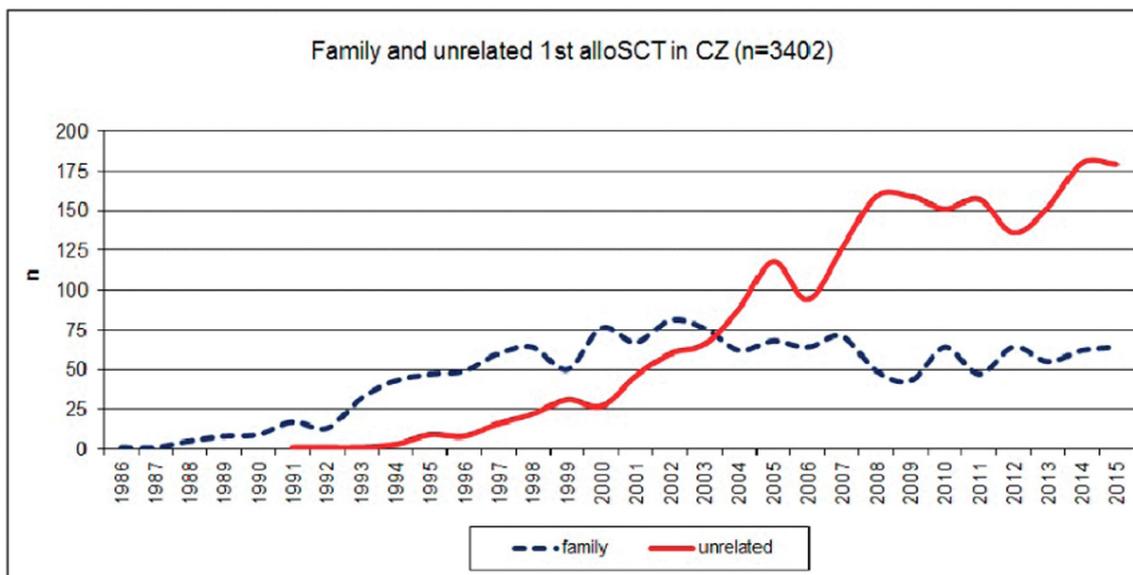
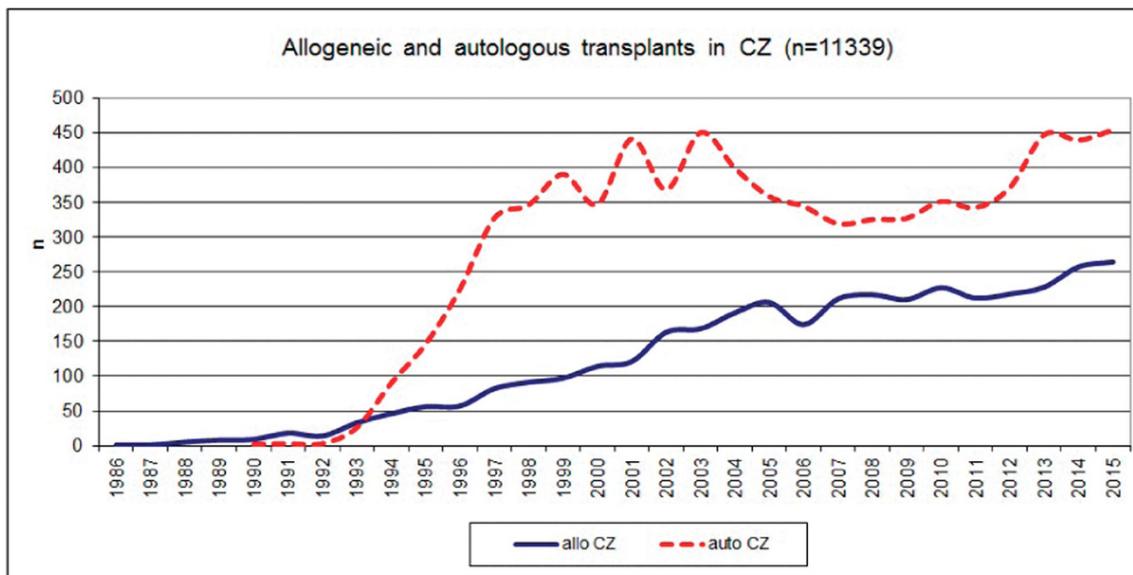
**P554**

**Transplant program evolution within 30 years and acute leukemia improved outcome**

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First stem cell transplant (SCT) in Czech Republic was performed in 1986. Altogether 11,399 SCTs were performed between 1986 and 2015. The aim of the current analysis is to describe indication evolution and transplant outcome of the whole transplanted population. Total nine centers are recognized as transplant centers. Allogeneic SCT (alloSCT) is performed in six centers, pediatric SCT is performed in two centers, only ASCT is performed in three centers, one center stopped the program in 2009 and the newest one started activity in 2013. EBMT reports, MED A and MED B, EBMT and Czech transplant database and EBMT transplant activity surveys were used as a source of information. Log-rank test was used for survival comparison. Out of 11,399 transplants performed between 1986–2015, 3699 were alloSCT (33%) and 7640 autologous (ASCT)(67%) (first ASCT was performed in 1990, graph 1). Transplant rate of ASCT is > 400/year/10 mil. inhabitants in last 3 years, alloSCT varies between 201 and 300/year/10 mil. inhabitants in last 9 years. According EBMT data, Czech Republic is among 15 countries in Europe with the highest frequency of alloSCT/10 mil. inhabitants. The most frequent indications for first alloSCT (n 3402) were AML (1092, 32%), MDS/MPN (560, 16%), ALL (526, 16%), CML (428, 13%), lymphomas (275, 8%), CLL (212, 6%), non malignant diseases (235, 7%) and others (74, 2%). The most frequent indications for 1st autologous transplants (n=6265) were: multiple myeloma and other plasma cell disorders (MM) (2264, 36%), NHL (2060, 33%), HL (602, 10%), solid tumors (839, 13%), AML/ALL (292, 5%), CLL (107, 2%) and others (91, 1%). The most rising diagnosis is alloSCT for AML which reached 250% in 2015 compared to 2005. Trends of the source of cells use, myeloablative and non myeloablative transplants (non-myeloablative became more frequent than MAB in 2004) as well as family and unrelated transplants (MUD became more frequent than SIB in 2003) are similar to European data (graph 2). We evaluated OS probability and death risk reduction of all transplanted patients in the two periods 2006–2015 (median age 53 y) vs 1990–2005 (median age 46 y). We observed death risk reduction for pts transplanted in the later period for alloSCT AML (median OS 43.2 vs 18.1 m, HR 0.755,  $P < 0.005$ ), alloSCT for ALL (median OS 53.2 vs 17.7 m, HR 0.842, ns), alloSCT for CLL (median OS 50.5 vs 36.9 m, HR 0.752, ns), alloSCT for lymphomas (median OS not reached vs 33.4, HR 0.771, ns), ASCT for MM (median OS 72.9 vs 65.6 m, HR 0.888,  $P = 0.069$ ). There was not observed any improvement in ASCT for NHL (median OS not reached vs 204 m, HR 0.972) as well HL (median OS not reached vs 196, HR 0.949, ns). The analysis of transplant program evolution within 30 years reveals important trends towards alloSCT increase including the use of alternative donors, changes in indications for alloSCT as well as for ASCT. Moreover better overall survival was observed in the majority alloSCT indications. The major shift was observed in AML both in increased number of alloSCT as well as most significant death risk reduction in the latest period. This unselected analysis demonstrates the success of the program and more detailed analysis of different subgroups is on the way.

**Disclosure of conflict of interest:** None.

#### P555

### Treatment of refractory pediatric acute lymphoblastic T cell leukaemia with anti-programmed cell death protein 1 (PD-1) antibody after allogeneic stem cell transplantation

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Children affected with acute lymphoblastic T cell leukaemia (T-ALL) and relapse after allogeneic stem cell transplantation (SCT) have limited treatment options and a poor prognosis. Immune checkpoint inhibitors targeting the programmed death (PD-1) receptor pathway may enhance the graft-versus-leukaemia (GvL) effect by blockade of inhibitory signals to T cells mediated by its ligand PD-L1. We report a 9-year old girl with refractory T-ALL after allogeneic SCT, who was treated off-label with the PD-1 inhibitor pembrolizumab. The girl was diagnosed with T-ALL (8.2 G/l WBC, 82% bone marrow infiltration, CNS negative, t (6;11)) and underwent HLA-haploidentical bone marrow transplantation from her mother with post-transplant cyclophosphamide since she failed to achieve molecular remission despite an intensified chemotherapeutic regimen. On day 100 post SCT, she had a 100% donor chimerism and decreasing minimal residual disease (MRD) marker (minimal  $1 \times 10^{-6}$ ). 140 days post SCT she had a molecular relapse with an MRD of  $5 \times 10^{-3}$  and a subsequent morphological relapse as well as mixed donor chimerism. Further treatment regimens included chemotherapy, intrathecal therapy and four donor lymphocyte infusions (DLIs). Initially, she displayed a good morphological response to DLIs but the leukaemic burden eventually remained stable with an MRD of  $2 \times 10^{-2}$ . Considering 54.2% PD-1 expression on CD3+ T cells in the patient's bone marrow and the encouraging data in other hematologic malignancies an off-label therapy with the PD-1 inhibitor pembrolizumab 1-4 was initiated. The patient and her parents gave informed consent and she received a single dose of pembrolizumab at 3.3 mg/kg 343 days after SCT. One week after administration of pembrolizumab, the patient developed acute GvHD grade IV of the skin, mucosa, liver, lung, central nervous system and eyes. She had a severe generalized inflammatory reaction with high inflammatory markers, increased hepatic transaminases and lymphocytic infiltration of the liver, cerebrospinal fluid and bronchoalveolar lavage fluid. Magnetic resonance imaging (MRI) of the brain revealed periventricular white matter lesions and hyperintensities of basal ganglia and bilateral temporal lobe consistent with autoimmune encephalitis. Treatment with high-dose corticosteroids, cyclosporine and the anti-interleukin 6 receptor antibody tocilizumab slightly improved her clinical condition. Her MRD value significantly decreased to  $4 \times 10^{-4}$  two weeks after administration and she achieved a 100% donor chimerism in bone marrow. Despite this promising response her medical condition deteriorated and the severe inflammatory reaction caused fatal multi-organ failure. This is to our knowledge the first report on a remarkable and fast response to PD-1 inhibition in a patient with pediatric T-ALL refractory to multiple lines of therapy including allogeneic SCT. This case illustrates the potential risk of checkpoint inhibitors to trigger severe GvHD that is not responsive to steroids. Induction of inflammatory GvL responses without causing severe GvHD by therapeutic checkpoint inhibition needs to be addressed in future clinical trials.

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**Disclosure of conflict of interest:** None.

P556

### Unmanipulated haploidentical-related donor HCT: A single center experience

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In recent years, there is a remarkable trend in the use of haploidentical-related hematopoietic cell transplantations (haplo-HCT) in patients who do not have a HLA matched related or unrelated donor. Here, we report our single-center experience, in patients who underwent haplo-HCT for acute leukemia. Between 2011 and 2016 seventeen consecutive adult patients, seven males and ten females, median age 42 years (range: 18–61 years) with high-risk acute leukemia underwent unmanipulated, BM or PBSC transplantation from an haploidentical family donor. Eleven patients transplanted for acute myeloid leukemia (5 in CR1, 1 in CR2, 1 in minimal active disease after CR1, 1 second trasplant in CR2, 1 transformed MDS in CR1, 2 AML secondary to myelofibrosis in CR1), 5 for acute lymphoblastic leukemia (2 in CR1, 3 in active disease) and 1 mastcell leukemia (secondary to AML) in active disease. Sixteen patients received myeloablative conditioning, and 1 reduced intensity, respectively. In five patients stem cells source was BM, in 12 were G-CSF mobilized PBSC. The median infused CD34+ cell dose was  $4.47 \times 10^6$  (range:  $1.0 \times 10^5$ – $8.2 \times 10^6$ ). Conditioning regimens were: BU-FLU-MAC (N=9), TBF-MAC (N=7), TBF-RIC (N=1) The regimens for GVHD prophylaxis were: PTCy as sole GVHD prophylaxis (N=1), MTX-CSA-ATG (N=9), MethylPred-ATG-Tacrolimus (N=6), ATG-CSA-MTX-MMF (N=1). Sustained trilineage engraftment occurred in 15 patients (88%), two patients died of transplantation-related complications before day 21 after transplantation without myeloid recovery. For patients receiving BM or PBSC grafts, the median time to >500 neutrophils recovery was 16 days (range: 10–36), and >20,000 platelets recovery was 16 days (range: 13–37). 7/15 patients (46.6%) and 2/15 (13.3%) had II–IV and III–IV grade of acute GVHD, respectively. The incidence of grade II–IV cGvHD was 27%. After a median follow-up of 11 months, 4/17 patients (23.5%), 4 out 5 patients transplanted in CR1, are alive and disease free at 50, 28, 19, 16 months (including the patient transplanted for AML after IMF). The 2-year probability of overall and progression-free survival was 40% (95% CI, 4.0–58.0%) and 28.6% (95% CI, 2.0–20.0%), respectively. Causes of death were: sepsis (N= 1), fatal aGvHD (N= 1), pneumonia (N= 1), toxicity (N=2), progression (N=4) and relapse (N=4). In our experience unmanipulated BM or PBSC transplantation from haploidentical family donor is feasible approach with high engraftment rates and acceptable TRM (23%) and rate of grade III–IV aGvHD, associated with durable remission in a proportion of patients with high-risk acute leukemia, specially in patients with AML transplanted in first remission.

**Disclosure of conflict of interest:** None.

## Aplastic anaemia

P557

### A feasible way to control infection by hematopoietic stem cell transplantation for children with aplastic anemia and refractory active infections

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It is generally recognized that allogeneic hematopoietic stem cell transplantation (allo-HSCT) should not be administrated to

patients with severe aplastic anemia (SAA) or very severe aplastic anemia (VSAA), when they got active infection. However, without neutrophil, severe infection is usually difficult to control and even fatal. Under these circumstances, rapid recovery of neutrophil by allo-HSCT might be an alternative to control infection. From January 2002 to December 2015, there were 175 young patients received allo-HSCT for SAA or VSAA at Shanghai Children's Medical Center in China. Among them, 22 patients (11 males and 11 females) with a median age of 7.0 years (range: 3.0–14.0 years) received allo-HSCT with refractory active infections. Refractory active infection was defined as persistent neutropenic fever with nonresponse to standard doses of broad-spectrum antibacterial agents and antifungal agents for more than three weeks, with or without definite focus of infection. Prior to allo-HSCT, four patients had persistent fever of unknown origin, 11 patients with single-site infection, and 7 patients with multiple-site infections. Sites of infection included lung, sinus, cellular tissue, peritoneum, liver, spleen and skin. The conditioning regimen consisted of fludarabine, cyclophosphamide and rabbit-antithymocyte globulin with or without total body irradiation (TBI) (2–3 Gy). Twelve patients were transplanted from mismatched unrelated donors, 3 from matched sibling donors, and 7 from haploidentical donors. Sixteen patients received G-CSF mobilized peripheral blood stem cells, three patients G-CSF mobilized peripheral blood stem cells plus G-CSF primed bone marrow stem cells, two patients bone marrow stem cells, and 1 patient umbilical cord blood stem cells. A median of  $11.4 \times 10^8$ /kg mononuclear cells with  $4.6 \times 10^6$ /kg CD34+ cells were transfused, except the patient who underwent UCBT with a total of  $1.3 \times 10^8$ /kg mononuclear cells and  $1.5 \times 10^6$ /kg CD34+ cells transfused. Eighteen patients achieved recovery of neutrophil and finally control of infections, including one patient who suffered primary graft failure and had autologous marrow recovery. Three patients died of infection and one patients died of acute renal failure before recovery of neutrophil. one patient died of pneumonia 8 months after allo-HSCT. one patient become thrombocytopenia after allo-HSCT. The other 16 patients are all disease-free. There were five patients developing grade I-II acute GVHD, and 4 patient grade III-IV acute GVHD. All were cured at last. three patients had localized chronic GVHD and one patient had extensive chronic GVHD. With a median of 2 years follow-up, the overall survival rate and disease-free survival rate are  $77.3\% \pm 8.9\%$  and  $71.3\% \pm 10\%$ , respectively. Allo-HSCT could be a feasible way to control infection for children with SAA or VSAA in the present of refractory active infections.

**Disclosure of conflict of interest:** None.

P558

### Aplastic anemia in the context of hemolytic paroxysmal nocturnal hemoglobinuria: intensive immunosuppression and eculizumab treatment. A retrospective analysis from two reference centers

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Paroxysmal nocturnal hemoglobinuria (PNH) may present hemolysis isolated (classical PNH) or associated with aplastic anemia (AA; AA/PNH syndrome). While classical PNH patients require anti-complement treatment (eculizumab), the treatment of AA/PNH patients should target their underlying AA by immunosuppression (IST), or even bone marrow transplantation (BMT). However, in a few patients clinically meaningful AA and hemolysis may be concomitant, eventually justifying both IST and eculizumab. To date there is no standard treatment for

this rare condition. Amongst a large cohort of 145 PNH patients (between 2007 and 2016) at our reference centers, St. Louis Hospital (Paris) and Federico II University (Naples), we retrospectively assessed characteristics and outcomes of patients diagnosed with AA/PNH who received intensive IST during or immediately before (3–6 months) eculizumab treatment. Nine patients were identified. Eight patients fulfilled the criteria of severe AA, and one had an immune-mediated isolated agranulocytosis. Since no patient had a HLA-matched related donor for BMT, all patients received intensive IST according to institutional guidelines. Six out of 9 patients were already on eculizumab treatment at the moment of starting intensive IST (concomitant treatment) whereas 3 patients received IST in the 3–6 months (median time of 3 months) before the introduction of anti-complement therapy (sequential treatment). For all patients already on treatment, eculizumab was not discontinued to minimize the risk of rebound intravascular hemolysis and thrombotic complications. Eculizumab was administered at the standard dose of 900 mg fortnightly in all but one patient, who needed an increased dose (1200 mg) because of pharmacokinetic breakthrough. Six patients (5 AA and 1 agranulocytosis), including the three undergoing a sequential treatment, received standard IST with horse-antithymocyte globulin (h-ATG, 40 mg/kg for four consecutive days) combined with cyclosporine A (CsA). The remaining three AA patients received alemtuzumab (3–10–30–30 mg subcutaneously in four consecutive days) and CsA within the prospective trial NCT00895739; one of these patients a few months later also received a second IST course with rabbit-ATG (3.5 mg/kg for five consecutive days) and CsA. All the patients completed the scheduled treatment without any side effect, including infusion-related reactions. Lymphocyte depletion ( $< 100/\mu\text{L}$ ) was observed in all patients irrespective of sustained therapeutic complement blockade. All the patients were available for response assessment at 6 months. Among the six patients receiving a concomitant treatment we observed one partial response (PR) and two complete responses (CR), whereas the three remain patients were non-responders (NR). Of them one was rescued with an unrelated BMT, while two remained on eculizumab treatment. One of the CR relapsed at 3 years showing clonal evolution and finally died. All the other patients are alive, keeping their hematological response. Patients receiving a sequential therapy were one in PR and two in CR 6 months after introduction of eculizumab. In conclusion, for patients diagnosed with severe AA/PNH syndrome intensive IST and eculizumab treatment, can be safely delivered either concurrently or sequentially, with an overall response rate of nearly 70%. This is the first systematic description of the management of severe AA in hemolytic PNH patients receiving eculizumab treatment.

**Disclosure of conflict of interest:** None.

#### P559

##### **Bone marrow transplantation for children with acquired bone marrow failure**

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Bone marrow transplantation (BMT) is a curative therapy for acquired bone marrow failure (aBMF) including aplastic anemia (AA) and hypoplastic myelodysplastic syndrome (MDS). It is difficult to differentiate AA from hypoplastic MDS in children. The 2008 WHO classification proposed a new entity in childhood MDS, refractory cytopenia of childhood (RCC). The

spectrum of patients with RCC is wide, ranging from patients with severe hypocellular bone marrow (BM) and mild dysplasia to those with normocellular BM and distinct dysplasia meeting the criteria for refractory cytopenia with multilineage dysplasia (RCMD) defined for adults with MDS. Until now, few studies have addressed the question whether the current WHO classification reflects clinical outcomes of BMT in childhood aBMF. Patients and We compared clinical outcomes of BMT among children with AA, RCC, and RCMD classified by the central morphology review of the Japanese Society of Pediatric Hematology and Oncology. Eighty-eight patients (male, 47; female, 41) including AA ( $n=26$ ), RCC ( $n=41$ ), and RCMD ( $n=21$ ) underwent BMT from March 2009 and August 2015. The median age (range) at the time of BMT was 11 (2–19) in AA, 12 (1–20) in RCC, and 8 (2–19) years in RCMD. Sixty-five patients underwent BMT from HLA-matched (related 41, unrelated 24) and 23 from HLA-mismatched (related 6, unrelated 17) donors. Conditioning regimens were used as follows: cyclophosphamide (CY)+antithymocyte globulin (ATG)  $\pm$  total body irradiation (TBI) ( $n=29$ ), fludarabine (FLU)+CY  $\pm$  ATG  $\pm$  TBI ( $n=42$ ), and FLU +melphalan (MEL)  $\pm$  ATG  $\pm$  TBI ( $n=17$ ). All patients got engraftment after BMT. However, late graft failure was found in 6 patients with RCC, and 7 with RCMD, but none with AA. Out of 13 patients who developed late graft failure, 8 patients used FLU+CY  $\pm$  ATG  $\pm$  TBI, 3 used CY  $\pm$  ATG  $\pm$  TBI, and 2 used FLU +MEL  $\pm$  ATG  $\pm$  TBI for conditioning regimens. Five-year cumulative incidence (CI) of graft failure was higher in RCMD ( $36 \pm 6.0\%$ ) than in AA (0%) and RCC ( $20 \pm 1.8\%$ ), significantly ( $P < 0.01$ ). Five-year CI of graft failure tended to be higher in FLU regimen ( $23 \pm 2.2\%$ ) than in CY+ATG  $\pm$  TBI regimen ( $10 \pm 0.65\%$ ), but not significant ( $P=0.20$ ). Five-year CI of graft failure did not differ between with ( $21 \pm 2.1\%$ ) or without TBI ( $29 \pm 6.3\%$ ) ( $P=0.57$ ). Multivariate analysis revealed that the morphological classification was a significant risk factor for graft failure ( $P < 0.01$ ). Five-year failure free survival rate ( $63 \pm 11\%$ ) in RCMD was significantly lower than in AA ( $96 \pm 3.8\%$ ) and RCC ( $74 \pm 7.9\%$ ) ( $P=0.02$ ). Graft failure, second malignancy, and death were considered as failure events. One patient with AA died of infection, four with RCC died of infection ( $n=2$ ), bleeding ( $n=1$ ) and myocarditis ( $n=1$ ), and one with RCMD died of infection. Five-year overall survival rates were not different among 3 groups (AA,  $96 \pm 3.9\%$ ; RCC,  $89 \pm 5.2\%$ ; RCMD,  $95 \pm 4.7\%$ ) ( $P=0.53$ ). High incidence of graft failure in RCMD may be due to higher BM cellularity than in AA and RCC. The optimal conditioning regimen of BMT should be established for children with aBMF based on the BM cellularity and morphological classification.

**Disclosure of conflict of interest:** None.

#### P560

##### **Decreased toxicity of allogeneic matched related donor bone marrow transplantation for pediatric patients with severe aplastic anemia using "low dose" cyclophosphamide, ATG plus fludarabine. A small pilot study and a new standard of care?**

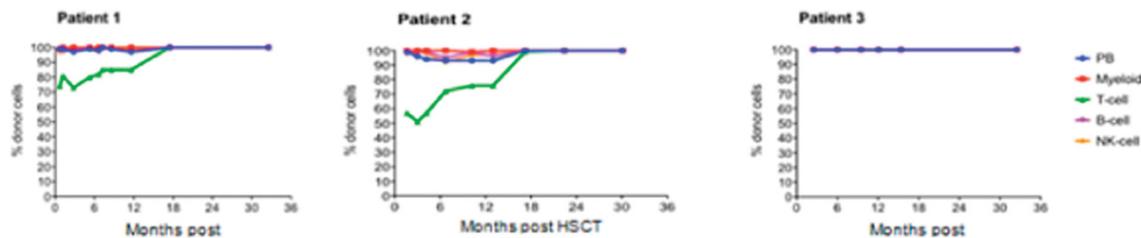
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The combination of cyclophosphamide (CY) and antithymocyte globulin (ATG) has been used as a standard conditioning regimen for matched related donor transplantation in patients with severe aplastic anemia (SAA). To decrease the regimen related toxicity (RRT) while maintaining appropriate engraftment and survival rates, fludarabine (FLU) was added to the regimen. Four pediatric patients received matched related donor bone marrow transplantation with CY (50 mg/Kg/2) [instead of the 50 mg/Kg  $\times$  4 standard dosing], equine ATG (30 mg/Kg  $\times$  4) with the addition of FLU (30 mg/m<sup>2</sup>  $\times$  4). GvHD prophylaxis included a calcineurin inhibitor and methotrexate. Patient characteristics are shown in Table 1. No grade 4 acute toxicities occurred during the first 30 days post transplant. All patients engrafted with

Table 1

Patient	Age (years)/ Sex	Initial counts				Previous Tx (No. units)	Time to HSCT (months)	Donor	CMV status donor/recipient	CD34+ X 10 <sup>6</sup> cells/kg	Engraftment Neut Pts	Last Tx RBC Pts	aGvHD/ cGvHD	Outcome
		ANC (x10 <sup>9</sup> /L)	Hb (g/dl)	Retic (%/d)	Plt (x10 <sup>9</sup> /L)									
1	13/F	0.4	8.6	39000	9	4	4.9	Brother	Neg/Pos	4.6 x 10 <sup>6</sup>	d+12 d+20	d+12 d+19	None	Alive 46 months
2	6/M	0.2	9	24000	6	3	0.7	Sister	Neg/Pos	5.2 x 10 <sup>6</sup>	d+14 d+19	d+17 d+19	None	Alive 42 months
3	11/M	0.7	7.2	24000	8	8	1.8	Sister	Neg/Neg	3.4 x 10 <sup>6</sup>	d+15 d+21	d+36 d+20	grade I-II/ cGvHD	Alive 41 months
4	20/F	0.1	8.6	14000	5	>20	4.1	Brother	Pos/Neg	5.3 x 10 <sup>6</sup>	d+17 d+27	d+20 d+25	None	Alive 34 months

Abbreviation: ANC = absolute neutrophil count; Hb = hemoglobin; Retic = reticulocyte count; Plt = platelet count; Tx = transfusion; Neut = neutrophil; RBC = red blood cell; aGvHD = acute graft versus host disease; cGvHD = chronic graft versus host disease



normalization of peripheral blood counts and transfusion independence (Table 1). One patient developed grade 1–2 acute graft-versus-host disease (GvHD) followed by chronic GvHD that resolved. With a median follow up of 41.7 months, all four patients are alive transfusion-free with complete donor chimerism (Figure 1–Pt 4 not shown; data only at intermittent time intervals). This combination of low dose CY/ATG+FLU regimen was better tolerated than high dose CY/ATG, and contributed to a successful outcome including engraftment, chimerism and survival. This small pilot study shows that this cytoreductive regimen could be considered as the standard of care for transplantation of patients with aplastic anemia from HLA-matched siblings.

**Disclosure of conflict of interest:** None.

### P561

#### First line treatment of aplastic anemia with thymoglobulin in Europe and Asia: A study of 976 patients treated 2001–2012

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Recent studies have suggested inferior outcome of patients treated with rabbit ATG (Thymoglobulin, Sanofi) as compared to horse ATG (ATGAM, Pfizer or Lymphoglobulin, Genzyme), and a higher early mortality with rabbit ATG has been suggested to explain this difference. Aim: To assess early mortality, response rates at 3, 6 and 12 months and long term outcome, in a large cohort of AA pts, treated in Europe or Asia with rabbit ATG and cyclosporin, as first line treatment. Eligible for this study were pts with AA, treated with Thymoglobulin between 2001 and 2012 in Europe (n=519) or Asia (n=457). Median year of treatment, was 2008; characteristics were comparable: median age 20 and 21 years, interval diagnosis treatment (23 and 25 days) and severity of the disease

(46% and 48% with vSAA). Early mortality was analyzed for all 976 patients. Long term outcome was also analyzed for 800 pts for who response data (no, PR, CR) were available. Mortality < 90 days was 5.5% and 2.1%, respectively, in the time period 2001–2008 and 2009–2012 (P=0.007). In these 2 time periods, early mortality for patients aged 0–60, was reduced from 3.5% to 1.4% and for patients over 60, from 22% to 9%. Overall response was recorded in 800 patients. At 6 months the cumulative incidence of response was comparable in the 2 time periods: 62% vs 66%, and at 1 year, 73% vs 75% (P=0.8). Response rates at 6 months were age dependent: 68%, 66%, 62%, 40% respectively in patients aged 0–20, 21–40, 41–60, >60 (P=0.0006). When non responders at 3 months were re-evaluated at 1 year, 59% had responded, 26% were non responders, 5% had died, and 10% had received other treatment. Responses at 6 months, were 60%, 66%, 74%, in pts with very severe, severe and non severe AA (P=0.0001). The actuarial 10 year survival for the entire population was 71%, and 70%, when pts were censored as surviving at transplant. Actuarial 10 year survival in univariate analysis was as follows: 89% vs 61% for day 90 responders vs non responders (P<0.01), 68% vs 80% for males versus females (P=0.07); 82%, 72%, 66%, 27% in pts aged 0–20, 21–40, 41–60, >60 years (P<0.001); 67%, 78%, 76% in pts with neutrophils < 0.2 x 10<sup>9</sup>/L, 0.2–0.5 x 10<sup>9</sup>/L and > 0.5 x 10<sup>9</sup>/L (P<0.001); 77%, 75%, 68% for pts with an interval diagnosis-treatment of < 30 days, 31–60 days or > 60 days (P=0.002). Finally pts treated >2008 had a 5 year survival superior to pts treated before 2008 (84% vs 77%, P=0.01). Survival at 5 years, in the recent period (2009–2012), was 83% for pts aged 1–60 and 60% for pts over 60 years. In multivariate Cox analysis the following variables remained independent predictors of survival: patient age, year of treatment, severity of the disease, interval diagnosis treatment, and gender. Thymoglobulin +CsA is effective and safe in patients with AA. The outcome is mainly age dependent. The interval between diagnosis and treatment remains a strong predictor: the earlier the better. For pts < 60 years old current early mortality, 4. For pts > 60 years of age, current early mortality is higher (9%), response rate (40%) and 5 year survival (70%) are lower. 5. The actuarial 10 year survival for the entire population was 71%. Survival at 5 years has improved from 77% (< /u>2008) to 84% (2009–2012), especially for pts over 60 years (37% vs 70%, P=0.006).

**Disclosure of conflict of interest:** We thank Centers for providing up date follow up of their patients . This study was supported by a grant of Sanofi-Genzyme.

#### P562

##### **Haploidentical hematopoietic stem cell transplantation with posttransplant cyclophosphamide for severe aplastic anemia complicated with infection**

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Very severe aplastic anemia (VSAA) patients with extremely low ANC have very low response rate to frontline IST and high early death rate of infection, hematopoietic stem cell transplantation and fast immunologic reconstitution may be the only salvage therapy for patients of VSAA with life-threatening infection. We describe haplo-identical HSCT with high-dose post-transplant cyclophosphamide (PTCy) in 2 cases of VSAA with persistent infection of paranasal sinuses. Case1: girl, 10 years, history of pancytopenia, persistent high fever, nasal congestion and swelling around the nose for 3 months. CT of paranasal sinuses demonstrated shadow of soft-tissue in both maxillary, ethmoidal and sphenoid sinuses. Histological examination of the mass of nasal cavity showed only necrotic tissue. Case 2: boy, 9 years, history of pancytopenia for 6 months, high fever and facial swelling around the nose for 6 weeks. He had a history of severe cholangiolitic hepatitis of unknown etiology about 5 months before the onset of VSAA. Both of the patients have extremely low ANC  $< 0.05 \times 10^9/L$ , Ret  $< 0.5\%$ , Plt  $< 20 \times 10^9/L$ . Both was given antibiotic treatment with carbapenem, vancomycin/linezolid, voriconazole or amphotericin B liposome and got no response. No pathogenic bacteria or fungus was found from either of the patients. Both of them had no full sibling or matched unrelated donor and had their father as their haploidentical donor. Bone marrow combined with peripheral blood stem cell (PBSC) was adopted. Conditioning: fludarabine days -5 through -2, (40 mg/m<sup>2</sup> × 4), intravenous busulfan (1.1 mg/kg q6h) on days -4 to -2. GvHD prophylaxis: high-dose cyclophosphamide 40 mg/kg on days +3 and +4, MMF and Tacrolimus since days +5. Rabbit anti-thymocyte globulin (Thymoglobulin) 2.5 mg/kg on days -4 to -2. Stable neutrophil engraftment (ANC  $> 0.5 \times 10^9/L$ ) occurred on day +13 and day+19 respectively. Platelet achieved  $20 \times 10^9/L$  on day +11 and day +55, respectively. Both transplant course was complicated by febrile neutropenia without detected etiology, while both children have no fever since the first day ANC  $> 0.5 \times 10^9/L$ . The facial swelling was resolved in both patients except for palatal fistula and fistula of maxillary sinus as the sequela of severe nasosinusitis. No acute or chronic GvHD. Case1 had hemorrhagic cystitis on day +30 which last for about 30 days, and suspected thrombotic microangiopathy (TMA) with hypertension, thrombocytopenia, elevated LDH and creatine on day +51 which was resolved soon after discontinuation of tacrolimus. Case2 had delayed engraftment of platelets and herpes simplex virus 6 encephalitis on days +40 which was cured by ganciclovir and high dose intravenous immunoglobulin. Now they are 9 and 7 months post-HSCT respectively and are doing well with 100% chimerism and no GvHD. Alternative donor HSCT may be considered as the first line salvage therapy for patients of VSAA with extremely low ANC and active infection. Haplo-identical HSCT make sure nearly every patients can find a donor. PTCy is proved to be efficient and safety in GvHD prophylaxis and facilitating engraftment in these two challenging cases.

**Disclosure of conflict of interest:** None.

#### P563

##### **Long-term outcome of patients with severe aplastic anemia receiving allogeneic hematopoietic cell transplantation using nonmyeloablative conditioning with fludarabine and low dose total-body irradiation**

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Allogeneic haematopoietic stem cell transplant (AHCT) offers the best prospect of cure in patients with severe aplastic anaemia (SAA). The use of myeloablative HCT is however limited by the toxicity of preparative regimens, the lack of matched sibling donors, transplant related mortality and graft rejection. The introduction of non-myeloablative (NM) conditioning offers the possibility of extending this potentially curative treatment to patients in whom AHCT was previously contraindicated. In 2006, we reported the outcome of 8 patients with SAA who have received AHCT using non-myeloablative conditioning comprising of 3 days of Fludarabine at 25mg/m<sup>2</sup> and total body irradiation at 2 Gy (Flu + TBI 2Gy). Here, we report a longer follow-up, with 6 additional patients who had received AHCT with this regimen. Fourteen patients with a median age of 37 years old (range: 17–48 years old) received Filgrastim-mobilised peripheral blood stem cell transplant from either HLA identical siblings ( $n=12$ ) or matched unrelated donor ( $n=2$ ) after receiving NM conditioning consisting of Flu + TBI 2Gy. The first two patients received Cyclosporine (CyA) and Mycophenolate Mofetil (MMF) for the post-transplant immunosuppressive therapy. The remaining 12 patients received CyA, MMF and a short course of Methotrexate (MTX) for additional graft-versus-host disease (GVHD) prophylaxis. Results All patients achieved prompt engraftment. The median time for engraftment of neutrophils ( $> 0.5 \times 10^9/L$ ) and platelets ( $> 20 \times 10^9$ ) were 16 days (range: 13–20 days) and 13 days (range: 8–25 days), respectively. Chimerism analysis on day 28 and subsequently showed  $> 95\%$  donor cells in all patients except 1, who developed secondary graft failure at 3 months and required salvage HCT. None of the patients experienced grade 3 and above regimen-related toxicity. Five patients developed grade II–IV acute GVHD and 2 patients developed limited chronic GVHD. With a median follow-up of 8.8 years (range: 0.54–14.52 years), the estimated overall survival and event free survival were 86% and 79% respectively. The two patients who did not receive MTX developed acute GVHD of the liver and succumbed to infective complications. The remaining 12 patients who had received triple immunosuppressive therapy were well, with limited chronic GVHD seen only in 2. Our results suggest that the NM conditioning regimen comprising of Flu + TBI 2Gy provides sufficient immunosuppression to allow prompt and stable engraftment with minimal regimen-related toxicity. It is an attractive option for patients with SAA who require AHCT but are at increased risk of regimen-related complications from more intensive cyclophosphamide-based regimens.

**Disclosure of conflict of interest:** None.

#### P564

##### **Paroxysmal nocturnal hemoglobinuria: A long-term single center experience**

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal disorder of hematopoietic cells characterized by the triad of hemolytic anemia, cytopenias and high risk of venous thrombosis. Due to the rarity of the disease, most reported

data derive from multicenter studies. We describe the natural history of the disease in a 30-year (yrs) long single center series of PNH patients (pts). We performed a retrospective analysis of 42 consecutive pts followed at our center from 1985 to 2016. Since 1985, the diagnosis was made by Ham test; starting from 2000, flow cytometry (FC) analysis was used to diagnose new pts and to confirm PNH in pts previously diagnosed by the Ham test. At diagnosis, 26 pts had classic PNH, 9 aplastic PNH and 7 intermediate form. The cumulative incidences of thrombosis, cytopenia and clonal neoplasm were 39%, 18% and 10%, respectively. Except for 1 pt with aplasia, no severe infections were diagnosed, nor renal failures or pulmonary hypertension. The 30 yrs overall survival (OS) was 84%. A non-significant better OS was associated to the absence of thrombotic events (96% vs 80%) and to a diagnosis made during the last decade (100% vs 90% vs 75%). Up to 2005 the treatment options were supportive care or allogeneic bone marrow transplantation. Since 2005, eculizumab was used in transfusion-dependent patients and/or in case of a thrombotic history. Overall, 14 pts were transfusion-independent for the entire period of the illness, 28 were transfusion-dependent and/or had thrombotic events (8pts). Six of the latter pts never received eculizumab but only transfusion support (3 pts) or allogeneic bone marrow transplant (3 pts), while 22 pts received eculizumab (the first 4 pts were included in the phase III TRIUMPH and SHEPHERD trials). Considering the increased risk of meningococcus infection for pts on eculizumab, vaccination with conjugated anti-meningococcus serotypes ACWY was employed and, since 2016, conjugated anti-meningococcus serotype B was added. Overall, 18 pts treated with eculizumab became transfusion-independent and four remained transfusion-dependent. No thrombotic event was observed after eculizumab, even if 8 pts had recurrent thromboembolisms prior to receiving the drug. No severe infection was documented. One patient developed extravascular hemolysis and receive a successfully selective splenic artery embolization. The 10 yrs OS in the eculizumab group was 92%. No PNH-associated death occurred. Our study confirms that thrombosis is a major complication in PNH pts not receiving eculizumab, influencing OS. The better OS in the last decade is probably due to the use of eculizumab and to lack of thrombotic events. In particular, for 22 pts on eculizumab the 10 year OS was 92%, even though half of the pts had thromboembolism and diagnosis made prior to the last decade. Although kidney failure and lung hypertension have been reported, we did not observe these complications in our long follow-up case series. We can assume that the availability of a dedicated emergency room at our Center allows to perform, promptly, hyper-hydration or transfusion support in case of hemoglobinuria crisis, reducing the risk or organ damage. No infections have been observed after eculizumab, probably due to the vaccination program schedule recommended in the literature, plus the addition of conjugated anti-meningococcus serotype B. However, shared guidelines are needed.

**Disclosure of conflict of interest:** None.

**P565**

**Transplantation in patients over 40 with acquired aplastic anemia: Mortality has not been reduced from 2010 to 2015**

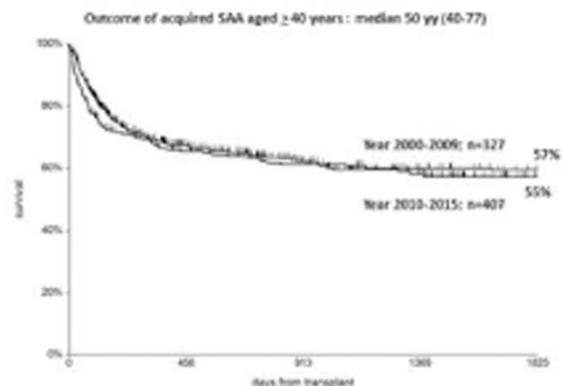
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Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking in to account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines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

whether this is still true in 2017. The aim of the study is to assess whether TRM in SAA patients grafted 2010–2015 is reduced, as compared to the era 2001–2009. We used the WPSAA EBMT registry, and identified 748 pts aged 40 years or more, with acquired SAA, grafted between 2001 and 2015. We divided pts in 2 transplant eras: 2001–2009 (n = 327) and 2010–2015 (n = 407). In the more recent period (2010–2015) pts were older (53 vs 49 year,  $P < 0.01$ ), were more often grafted from alternative donors (ALT) (64% vs 43%,  $P < 0.01$ ), with a greater use of BM (54% vs 41%,  $P < 0.01$ ), and with a longer interval dx-tx (317 vs 258 days,  $p < 0.01$ ), and more often received a fludarabine containing regimen (55% vs 42%,  $P < 0.01$ ). The OS 5 year of pts grafted in 2001–2009 was 57%, compared with 55% for pts grafted 2010–2015 ( $P = 0.7$ ). In multivariate analysis, including the interval diagnosis transplant, patient's age, donor type, stem cell source and conditioning regimen, the lack of improved survival in 2010–2015 was confirmed ( $P = 0.3$ ). A very strong age effect was shown both in univariate and multivariate analysis: survival of pts aged 40–50 years, 51–60 years and  $> 61$  years, was respectively 64%, 54%, 41% ( $P < 0.0001$ ) and this was confirmed in multivariate analysis. The conditioning regimen, also proved to be a significant predictor, with improved survival for ALT transplants receiving FLU containing regimens (56% vs 46%,  $P < 0.001$ ). In general pts receiving either CY200 or a FLU containing regimen, did significantly better than pts receiving other preparative regimens (58% vs 50%,  $P = 0.02$ ). The use of a sibling donor (SIB) did not prove to predict survival in multivariate analysis. Pts receiving Campath in the conditioning, did significantly better than pts not receiving Campath (65% vs 54%  $P < 0.01$ ); similarly survival of patients with ATG was superior 59% vs 41% compared to patients not receiving ATG ( $P < 0.01$ ). When pts receiving either Campath or ATG (n = 564) were compared to patients not receiving either (n = 161), the difference in survival was 61% vs 41% ( $P < 0.0001$ ), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods ( $P = 0.02$ ), acute GvHD grade II–IV was reduced from 15% to 11% ( $P = 0.1$ ) and chronic GvHD was also reduced from 32% to 26% ( $P = 0.04$ ). Infections remain the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%), whereas PTLD have been reduced from 3% to 0.5%. HSCT in pts with acquired SAA aged 40 and over, continues to carry a significant risk of TRM also in 2010–2015, ranging from 36% in younger pts (40–50) to 59% in older pts ( $> 60$  years). Survival is predicted in multivariate analysis, by two crucial predictors: patients' age and the use of either Campath or ATG, the latter giving a 20% survival advantage over no Campath/ATG. ALT and SIB donors produce similar survival. This study gives further support to current guidelines, suggesting first line therapy with ATG+CsA, in pts over the age of 40.

[P565]



**Disclosure of conflict of interest:** None.

### P566

#### Allogeneic haematopoietic stem cell transplantation as curative therapy for early-onset, refractory Crohn's disease

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Results of a recent randomized trial suggest that autologous HSCT is an option in adult patients with severe, therapy-refractory Crohn's disease (CD) with an associated mortality risk of 4%. However, relapse of the disease is frequent (1). In contrast allogeneic HSCT has resulted in long-term cure of CD in affected patients transplanted because of haematological malignancy (2). We report a 17 year old girl who was diagnosed with severe CD at age seven (Paris classification L3, L4a, B1). Neither next generation sequencing nor immunological work up identified a monogenetic cause of CD. Progressive chronic inflammation manifesting ubiquitously in the gastrointestinal tract resulted in severe complications, such as perianal fistulas with rectal stenosis, intestinal abscesses, dysphagia, severe weight loss, failure to thrive, delayed puberty and the need for ileostomy and long-term exclusive enteral nutrition via tube feeding. Despite multiple lines of therapy, including repeated nutritional therapy, steroids, immunosuppressants (methotrexate, azathioprine) and biologicals (infliximab, adalimumab, certolizumab) a lasting remission could not be achieved resulting in poor quality of life. After careful risk/benefit assessment including ethical counselling allogeneic HSCT was offered. She underwent allogeneic HSCT from a matched (10/10) unrelated bone marrow donor ( $4.3 \times 10^8$ /kg total nuclear cells). Conditioning was performed according to a protocol successfully applied in adolescents with chronic granulomatous disease (3) with alemtuzumab ( $3 \times 0.2$  mg/kg/d), targeted busulfan (tAUC 53770 ng × h/ml) and fludarabine ( $6 \times 30$  mg/m<sup>2</sup>). Cyclosporine A and mycophenolate mofetil were used as GvHD prophylaxis. Neutrophil and platelet engraftment were observed on days +20 and +24, respectively. The post HSCT course was complicated by grade I acute skin GVHD treated with topical steroids and toxic megacolon secondary to scarring stenosis on both ends of the unused colon on day +130 requiring surgery and a colectomy. At 12 months post HSCT the patient is well, off immunosuppressive medication, without GVHD and exhibiting >95% donor chimerism. The CD is in complete clinical and histological remission as proven by endoscopy and biopsies. Stoma reversal with restitution of intestinal continuity is planned for the next 12 months. Refractory CD can lead to life-threatening complications and severely reduced quality of life. Although long-term outcome in our patient will need to be carefully assessed, allogeneic HSCT may offer a curative therapy in children and young adults with severe CD, even in the absence of an identified monogenetic cause.

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**Disclosure of conflict of interest:** None.

### P567

#### Autologous haematopoietic stem cell transplantation (AHSCT) for Crohn's disease (CD): A retrospective study of outcomes from the EBMT autoimmune diseases working party (ADWP)

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Current EBMT recommendations include consideration of AHSCT in exceptional circumstances for patients with severe refractory CD. The only randomised trial of AHSCT in CD (ASTIC) confirmed substantial short-term benefits but failed to meet its primary 1 year endpoint. To further clarify the long-term safety and efficacy of AHSCT in CD we performed a retrospective analysis of patients undergoing AHSCT for CD outside the ASTIC trial using the EBMT registry. Patients were identified from the EBMT registry. All adult patients undergoing AHSCT for a primary diagnosis of CD from 1997 to 2015 were eligible for inclusion. Patients who were enrolled in the ASTIC trial were excluded. From a total of 99 patients (across 27 centres) on the EBMT registry, data were obtained from 76 patients transplanted in 14 centres in 7 countries. Median patient age was 30 yrs (range: 20–51) and 63% were female. Median age at first diagnosis of CD was 18yrs (range: 2–48). Patients were heavily pre-treated, having failed or been intolerant to a median of 6 previous lines of therapy (range: 3–10). 55% had received experimental therapy prior to auto-HSCT. 80% of patients had undergone at least 1 operation. The median time from first diagnosis of CD to auto-HSCT was 12.3 years (range: 1.3–25.8). All patients received peripheral blood stem cells following conditioning with cyclophosphamide 200 mg/kg and 84% received anti-thymocyte globulin (ATG). The median CD34+ dose infused was 5.5 (range: 2.4–40.6) × 10<sup>6</sup>/kg. Twelve percent of patients underwent CD34+ selection. Neutrophil and platelet engraftment occurred at a median of day 10 (range: 6–18) and day 9 (range: 1–44), respectively. Sixty-one percent received post transplant G-CSF. Median length of follow-up following auto-HSCT was 42 months (range: 6–174). At 100 days post auto-HSCT, 64% of patients were in clinical remission (CR), defined as no abdominal pain and normal stool frequency. A further 27% experienced significant improvement, defined as improvement in abdominal pain and stool frequency. For 5% there was no appreciable change in disease and in 4% the disease worsened compared to baseline. At 1 year post auto-HSCT, 39% were in CR, 19% were improved, 20% were unchanged and 22% had worsened. At last follow-up, 37% were in CR, 23% were improved, 25% were unchanged and 15% had worsened. Overall 74% restarted medical therapy post auto-HSCT and 38% required further surgery. Overall 26% developed an infection requiring treatment post auto-HSCT (11% bacterial, 12% viral). EBV and CMV reactivation occurred in 7% and 4% respectively and herpes zoster occurred in 4%. A secondary autoimmune disease developed in 13%, most commonly thyroid disease (63%). Malignancy developed in 5%, of which skin cancer accounted for 75% of cases. One patient died at 56 days post auto-HSCT due to CMV infection, sepsis and multiorgan failure. This large retrospective series further supports the safety and efficacy of AHSCT in a population with severe and treatment-refractory Crohn's disease, 60% of patients experienced complete remission or significant improvement in CD symptoms with long-term follow-up. TRM observed was similar to AHSCT for other indications. In summary, AHSCT appears to be an extremely promising therapy for severe refractory CD. Further follow up of ASTIC patients and future randomised trials are warranted.

**Disclosure of conflict of interest:** None.

### P568

#### CD4+ memory stem T cells: Novel players in Rheumatoid arthritis

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Memory Stem T cells (TSCM) are long living self-renewing memory T cells with long-term persistence capacity, which play a relevant role in immunological memory and protection against infectious diseases and cancer<sup>1,2,3,4,5,6</sup>. The aim of this work is to investigate the potential role of TSCM as a reservoir of arthritogenic T cells in Rheumatoid Arthritis (RA). We analysed the dynamics of circulating TSCM (here identified as CD45RA+ CD62L+ CD95+ T cells) and other memory T-cell subpopulations by multiparametric 11-color flow cytometry in 27 patients with active RA and in 14 of them also during treatment with anti-TNF $\alpha$  biological agents (Etanercept). To analyse cytokine productions, functional assays were performed stimulating peripheral blood mononuclear cells (PBMCs) with PMA/Ionomycin and Brefeldin A. After the stimulation, cells were stained for surface markers, fixed and stained for intracellular cytokines. We traced circulating antigen specific CD4+ T cells for the vimentin-derived citrullinated peptide (VimCit) 65SAVRAcitSSVPGVR77 7,8 in HLA-DRB1  $\times$  04:01 RA patients before and during the anti-TNF $\alpha$  treatment using custom MHC Class II Tetramers. Viral antigen specific CD8+ T cells were traced using MHC Class I Dextramers. Age-matched healthy donors (HDs) were used as control for all the experiments. We found a significant expansion of CD4+ TSCM in patients with active RA both in terms of frequency and absolute counts. Notably, CD4+ TSCM significantly contracted upon anti-TNF $\alpha$  treatment, suggesting a role of TNF $\alpha$  in TSCM accumulation. In contrast to CD4+ T cells, CD8 compartment did not show significant alterations compared to (HDs). Furthermore, CD4+TSCM in RA patients displayed an enrichment in the TH17 phenotype, largely implied in autoimmune disorders, while the other T cell subpopulation were not enriched in the TH17 phenotype. At the antigen specific level, we were able to trace in HLA-DRB1  $\times$  04:01 patients antigen specific CD4+ T cells, comprising TSCM, specific for the vimentin-derived citrullinated peptide. Of notice, citrullinated vimentin specific CD4+ T cells, including TSCM, contracted during anti-TNF $\alpha$  administration, while viral-specific CD4+ T cells (EBVBHRF-1) and antiviral CD8 specificities (CMVpp65, FluMP, EBVBMLF-1) were not affected by Etanercept administration, thus suggesting a possible role of CD4+ TSCM as reservoir of arthritogenic autoreactive T cells. Overall, our results suggest that TSCM, by representing a long-term reservoir of undesired specificities, might play a non redundant role in sustaining RA and possibly other T cell mediated disorders, thus representing novel biomarkers as well as therapeutic targets. Ongoing experiments will characterize the TCR repertoire on sorted TSCM and CD4+ memory subsets in order to identify a possible oligoclonality in TSCM repertoire. In conclusion, the analysis of TSCM dynamics in autoimmune disorders could have relevant clinical implications as new biomarkers and for devising innovative therapeutic strategies.

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**Disclosure of conflict of interest:** None.

#### P569

##### **EBV and CMV reactivation following autologous haematopoietic stem cell transplantation (HSCT) for autoimmune neurological diseases resolves spontaneously and rarely requires treatment**

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Autologous haematopoietic stem cell transplantation (HSCT) for severe autoimmune diseases involves immunosuppressive conditioning regimens and current guidelines recommend monitoring for viral reactivation of Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) (Snowden *et al* 2012). However, the incidence, degree and management of viral reactivation are not established. We performed a retrospective observational service evaluation study of all patients receiving cyclophosphamide 200 mg/kg + rabbit anti-thymocyte globulin 6 mg/kg (ATG, Thymoglobulin) followed by autologous HSCT for various autoimmune neurological diseases between 2011 and 2016 at our centre. Data collected included the baseline serological status of the patient prior to transplant and serial blood PCR quantitation (copies/mL). If EBV and CMV reactivation occurred details of further management was collected and descriptive statistics were used to summarise outcomes. Twenty-three patients received autologous HSCT between January 2011 and October 2016; 21 patients with Multiple Sclerosis (MS), 1 with Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) and 1 with Stiff Person Syndrome. Twenty-two patients had positive EBV IgG serology prior to transplant and 1 patient had an equivocal result. Seventeen patients had evidence of EBV reactivation and a further patient had EBV DNA detected post-transplant but with less than 250 copies/mL. The average time to peak EBV PCR was 26.5 (range: 12–44) days post-transplant and a range: in EBV PCR peak level from 623 to 577 000 copies/mL. The 4 patients who had EBV PCR results of over 100 000 copies/mL had CT scans of chest, abdomen and pelvis performed which did not demonstrate significant lymphadenopathy or hepatosplenomegaly. In all patients monitored for a detectable EBV reactivation, the EBV PCR spontaneously began to fall within 2 months (average 36 days, range: 18–60 days) post-transplant and no specific treatment was required. One patient had late EBV reactivation of 3480 copies/mL at 6 months post-HSCT associated with chronic tonsillitis and tonsillectomy specimens showed follicular hyperplasia without evidence of post-transplant lymphoproliferative disorder (PTLD) and EBV PCR levels normalised without other treatment. 8 (35%) patients had positive CMV IgG serology prior to transplant and one patient had an equivocal result. Only 1 of 23 patients had a significant reactivation of CMV with 51 300 copies/mL at 21 days post-transplant, successfully treated with intravenous immunoglobulins and valganciclovir. Two other patients had low level CMV reactivation with 94 and 476 copies/mL, respectively which resolved spontaneously without treatment. EBV reactivation in patients with neurological autoimmune disease undergoing autologous HSCT is common and usually resolves spontaneously without treatment. Asymptomatic CMV reactivation occurs in approximately 13% of patients in this setting and may require treatment.

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#### P570

##### **Exacerbation of multiple sclerosis symptoms in patients undergoing autologous stem cell transplantation**

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Autologous hematopoietic stem cell transplantation (HSCT) has been utilised for the treatment of severe multiple sclerosis (MS). It results in significant improvement of neurological function, although patients can experience exacerbations of MS-related symptoms during the procedure. We reviewed 17 patients with MS who underwent stem cell mobilisation and collection from March to November 2016. The median age was 40 years (24–55). Nine patients (53%) were male. The interval from diagnosis to HSCT was 114.3 months (range: 11.6–128.3). 9 patients (53%) had relapsing-remitting (RRMS), six patients (35%) secondary-progressive (SPMS) and two patients (12%) primary-progressive (PPMS) multiple sclerosis. Only 2 patients (12%) had not received any prior treatment, whereas 10 patients (59%) received two prior treatments, three patients (17%) received three treatments and two patients (12%) received four treatments. The median Expanded Disability Status Scale (EDSS) score was 6 (range: 2–6). Peripheral blood stem cells were mobilised with cyclophosphamide (CY) 2 g/m<sup>2</sup> on day +1 and daily GCSF (5 µg/kg subcutaneously) from day +3 until the completion of the harvest. HSCT was performed at a median of 33 days after mobilisation (range: 25–59). The conditioning regimen consisted of CY (50 mg/kg/day from day –5 to –2) and ATG (2 mg/kg/day from day –4 to –2). Exacerbation of MS symptoms was defined as the appearance of new or worsening of old symptoms for at least 24 h duration in a previously stable (4 weeks) patient. Of the total cohort, 13 patients (76%) underwent mobilisation with CY+GCSF uneventfully. Only two patients (12%) had an exacerbation of MS requiring hospital admission after collection (one with fatigue and increase of spasticity, other with worsening weakness). No patient required hospital admission during the mobilisation procedure. The median CD34+ cell dose was 8.39 × 10<sup>6</sup>/kg (range: 2.2–24). The median number of apheresis was 1(1–2). A total of 14 patients have undergone HSCT at the time of this analysis. During transplant a total of 11 patients (78%) experienced an exacerbation of MS. Of these, 54% (n=6) before day 0 and 46% (n=5) between day +4 and +7. Symptoms of exacerbation were: muscle spasms in 4 patients (36%), weakness and reduced power of limbs in 4 patients (36%), increase instability and tremor in two patients (18%) and one patient (10%) with worsening of neuropathic pain. Only three patients (28%) received treatment with methylprednisolone for MS exacerbation and symptoms had fully resolved by discharge in all patients. Other transplant complications included neutropenic fever in all, invasive fungal infection in 1, fluid overload in 9 (64%) and ATG related complications in 11 (78%) such as fever (n=10) and pericarditis/serositis (n=1). The median time to neutrophil engraftment was 10 days (10–14) and the median duration of hospital admission was 20 days (15–25). Exacerbation of MS symptoms is common during HSCT and can also occur during mobilisation. In our hands, after CY and GCSF mobilisation only two patients (11%) developed an exacerbation of MS symptoms compared with 11 patients (78%) after CT and ATG conditioned HSCT. It is possible that the addition of ATG to CY triggers an immunological response involved in this transient deterioration of the MS symptoms. Further studies are required to confirm this hypothesis.

**Disclosure of conflict of interest:** None.

#### P571

##### **Inflammatory immune response after autologous transplantation in neurologic diseases**

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Inflammatory immune response syndrome (IRIS) is a non-infectious worsening of neurological condition during immune

recovery and has been documented to occur in HIV and in multiple sclerosis following alemtuzumab. The manifestation of IRIS includes headache, nausea, weakness, neurologic deficits, and MRI enhancing lesion. We report three cases of IRIS after autologous non-myeloablative hematopoietic stem cell transplantation (HSCT) in patients for which the transplant indication was an inflammatory neurologic disease: Neuro-myelitis Optica (NMO), Chronic Relapsing Inflammatory Optic Neuritis (CRION), and Multiple Sclerosis (MS). Mobilization was with cyclophosphamide 2 gr/m<sup>2</sup> and GCSF. Conditioned regimen was 200 mg/kg cyclophosphamide (50 mg/kg/d) and 6.0 mg/kg rATG (Thymoglobulin). The conditioning regimen for NMO and CRION also included 1000 mg rituximab. Case 1. A 22 years old African-American female with Systemic Lupus Erythematosus (SLE) and NMO was discharged day 10 and readmitted on day 14 for fever, headache, progressive altered mental status with dysarthria and legs. Brain MRI had numerous T2/FLAIR hyperintense and enhancing lesions in the subcortical and periventricular white matter. A lumbar puncture was negative for infection including JCV. Complete recovery occurred after treatment included high dose of steroids and plasmapheresis. Case2. A 48 years old female with CRION experienced blindness, weakness and slurring of speech three months post HSCT. MRI showed a large enhancing brain stem lesion. Lumbar puncture was JCV negative. Complete recovery occurred after solumedrol and rituximab. MRI 6 months later demonstrated complete resolution of the enhancement with return of vision to baseline. Case3. A ten year-old boy diagnosed with paediatric MS developed hemichorea seven days after HSC reinfusion. Brain MRI revealed a gadolinium-enhancing lesion in the contralateral basal ganglia. Lumbar puncture was negative for infection including JC virus. Symptoms resolved spontaneously after seventeen days. The appearance of new neurologic symptoms and MRI enhancing lesions early after autologous HSCT is unexpected and may be related to lymphocytes in the graft, immune recovery post engraftment, and/or persistent auto-antibodies. It is mandatory to perform a lumbar puncture to exclude the possibility of infections including progressive multifocal leukoencephalopathy (PML) due to JCV. The timing of presentation, the negativity of JC viral load, and the complete recovery with or without immune suppression suggest the hypothesis of IRIS, as an epiphenomenon of the immune reconstitution following autologous HSCT for neurologic diseases

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#### P572

##### **Long-term outcomes and late effects at children with multiple sclerosis and neuromyelitis optica after hematopoietic stem cell transplantation**

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Hematopoietic stem cell transplantation is the effective method of therapy for CNS autoimmune disorders in children. Long-term outcomes and late effects estimation required. The aim of the study is to estimate long-term outcomes and late effects at children underwent Auto-HSCT for multiple sclerosis (MS) and Allo-HSCT for neuromyelitis optica (NMO). Twelve pts. with MS and 3 pts. with NMO were included to the analysis. MS pts. gender: female –75% (n=9), male –25% (n=3). All pts. with NMO were females. MS pts. median age –16.8 ± 1.6 years Median length of MS prior to HSCT –17.5 ± 3.4 months. Age of MS debut: 13.4 ± 1.5 years (4–15 years). Median EDSS 6.16 ± 0.2. All patients had severe refractory MS treated with corticosteroids, interferons, plasmapheresis and mitoxantron with negative results. All MS pts.

had inflammation signs. NMO pts. median age  $-12.7 \pm 1.4$  years Median length of NMO prior to HSCT  $-19.2 \pm 4.1$  months. Age of NMO debut:  $10.2 \pm 0.4$  years (9–11 years). All patients had severe refractory NMO treated with corticosteroids, interferons, plasmapheresis and rituximab with negative results. All NMO pts. had inflammation signs. Allo-HSCT for NMO patients performed due to life-threatening diseases course. Procedures for MS pts.: PBSC mobilization—Cph 60 mg/kg followed by G-CSF. Conditioning: Cph 200 mg/kg and ATGAM 160 mg/kg. PBSC reinfusion—on day 0. G-SCF stimulation from day +5. Conditioning for NMO pts.: Treo 42 gr/sq.m., Flu 150 mg/sq.m., Rituximab 375 mg/sq.m. and ATGAM 90 mg/kg for MUD. Late effects estimation due to developed protocol for late effects estimation at patients with HSCT for AD (somatic and neurocognitive status estimation). All pts. with MS showed the fast improvement (EDSS improvement: first 60 d.  $-3.1 \pm 0.3$ , after 60 d.  $-0.2 \pm 0.05$ ). Maximal EDSS improvement  $-5.5$ . EDSS at 2 pts. improved to 1. Median follow-up  $40.7 \pm 2.4$  months (10–76 months). Pts. with severe refractory secondary-progressive MS with the long duration of ineffective treatment (late HSCT) showed the minor response. Two pts. relapsed (clinical and MRI). No severe complications were registered. One pt. with NMO died due to refractory ADV-infection, two pts. did not have any severe toxic episodes. Median follow-up  $23 \pm 4.7$  months (1–60 months). Pts. with NMO stopped neurological progression and improved significantly after HSCT. MS pts. Late effects: cardio-vascular  $-5$  pts., endocrine  $-3$  pts. (all females). NMO pts. late effects: cardio-vascular  $-2$  pts., late immune reconstitution  $-1$  pt. One NMO pt. experienced skin cGVHD. All pts. have deficit in neurocognitive sphere and received special rehabilitation. HSCT is an effective way of autoimmune inflammation reduction and successful approach to the treatment of refractory pediatric MS and NMO. In-time Auto-HSCT can significantly improve the outcome of pediatric MS. Most MS pts. remain in remission during the long time of follow-up. Late effects after this treatment are cardio-vascular pathology, endocrine dysfunction. Allo-HSCT can be effective life-saving method for patients with NMO. Infections after Allo-HSCT can threat pts. with NMO. Late effects for NMO pts. include late immune reconstitution and cardio-vascular pathology. Both MS and NMO patients require active neurocognitive rehabilitation.

**Disclosure of conflict of interest:** None.

## Chronic leukaemia

P573

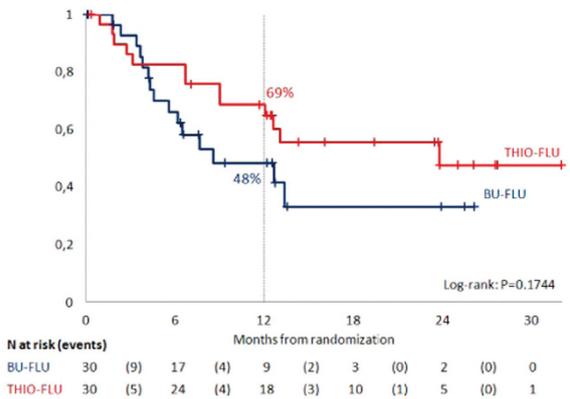
### Busulfan-fludarabine versus thiotepa-fludarabine as a reduced-intensity preparative regimen for allogeneic haematopoietic stem-cell transplantation in patients with myelofibrosis: A phase II, multicentre, randomized trial

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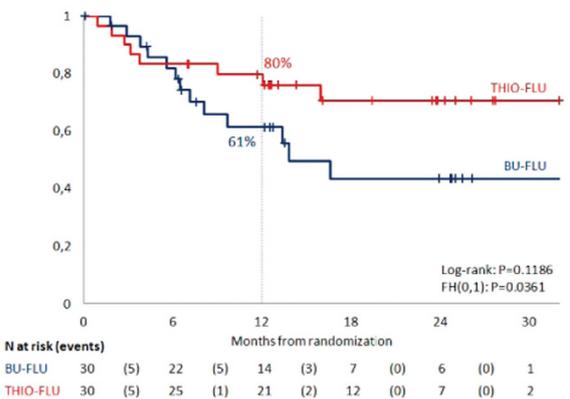
Allogeneic haematopoietic stem cell transplantation (HSCT) remains the sole curative option for patients with myelofibrosis (MF). Although a spectrum of conditioning regimens has been used, the optimal preparative treatment before HSCT remains to be defined. We did a phase II randomized study at 21 transplant centers in Italy with the aim of comparing the reduced-intensity conditioning (RIC) fludarabine-busulfan (FB) (conventional arm), that had been already tested in a

[P573]

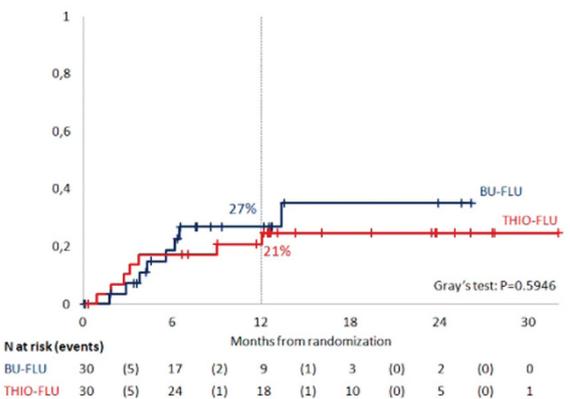
#### Progression Free Survival by treatment (ITT)



#### Overall Survival by treatment (ITT)



#### Non Relapse Mortality by treatment (ITT)



prospective EBMT study (1) with the RIC fludarabine-thiotepa (FT) (experimental arm), that has been widely used in Italy in the last two decades (2). Eligible to this study were patients with primary MF or a MF subsequent to a previous Essential Thrombocytemia or Polycythemia Vera, an age  $\geq 18 \leq 70$  years, a Karnofsky performance status  $> 60$ , a comorbidity index  $< </u> 5$  and with at least one of the following unfavorable prognostic factors: anemia (Hb  $< 10$  g/dL), leukocytosis ( $25 \times 10^9/L$ ), circulating blasts  $> 1\%$  or constitutional symptoms. Patients were randomized to receive intravenous busulfan 0.8 mg/kg for 10 doses or thiotepa 6 mg/kg for two doses associated to fludarabine 30 mg/m<sup>2</sup> for

six consecutive days. Anti-thymocyte immunoglobulin at 7.5 mg/kg total dose was administered in case of unrelated donors. From July 2011 to November 2015, 62 patients with a median age of 56 years (36–66) were enrolled into the study. DIPSS prognostic score was intermediate-1 in 21 patients (34%), intermediate-2 in 37 patients (60%) and high in 4 patients (6%), whereas DIPSS Plus score was intermediate-1, intermediate-2 and high in 15%, 45% and 40% of patients, respectively. Because of AML transformation detected at study entry, two patients were excluded from the final analysis. On an intention-to-treat basis, the primary study endpoint was the Progression Free Survival (PFS) at 1 year after transplantation. Donors were HLA-identical sibling (26), HLA-matched unrelated (26) or mismatched for a single class I HLA allele (10). Patients and donor characteristics in the two cohorts were similar. At day +30 after HSCT, 52 out of 57 evaluable patients (91%) engrafted, without significant differences according to donor type. With a median follow-up of 16 months of patients alive, at 1 year the following outcomes were observed in the FB vs. the FT arm: the PFS arm was 48% vs 69%, [HR 0.60 (95% CI 0.28–1.27)  $P=0.17$ ], the OS was 61% vs. 80% [HR 0.50 (95% CI 0.21–1.21)  $P=0.13$ ] and the NRM was 27% vs. 21% (Gray's test  $P=0.59$ ). Fourteen out of 57 evaluable patients (25%) developed grade II–IV acute GVHD, that was grade III–IV in only 4 patients (7%). By univariate analysis a lower 1-year PFS was observed in patients with DIPSS score intermediate-2 and high [HR 2.36 (95% CI 0.95–5.83)  $P=0.06$ ] and in patients receiving a transplant from unrelated donors [HR 1.99 (95% CI 0.90–4.41)  $P=0.09$ ]. No matter the conditioning regimen, NRM and disease progression remain open issues for patients with MF undergoing HSCT from both HLA-identical sibling and unrelated donors.

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**Disclosure of conflict of interest:** None.

#### P574

##### Impact of liver stiffness measured by transient liver elastography (FibroScan) on liver toxicity in myelofibrosis patients undergoing allogeneic stem cell transplantation

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Myelofibrosis (MF) is a hematologic malignancy which is characterised by extramedullary hematopoiesis due to bone

marrow fibrosis resulting in spleno-and/or hepatomegaly. Allogeneic stem cell transplantation (allo-HSCT) is the only curative treatment for MF but is associated with therapy related morbidity and mortality. Retrospective studies suggested an increase of liver toxicity in MF patients in comparison to other diseases following allo-HSCT. The aim of this prospective study was to evaluate the impact of liver stiffness measured by transient elastography (FibroScan) on liver toxicity after allo-HSCT. Between 2013 and 2015 we included 39 patients (male 64%, female 36%) who underwent allo-HSCT due to primary MF(72%), postPV/ET-MF (23%) or MF in transformation (5%). The median age of the patients was 62 years (range: 35–74). Conditioning regimen was mainly Busulfan based reduced intensity. All patients received ATG. GvHD prophylaxis was CsA/MMF in all patients. Stem cell source was peripheral blood in 95% and bone marrow in 5% of the patients. Donor sources were as follows: MRD (18%), MUD (77%) and haploidentical relative (5%). FibroScan was performed prior to conditioning. Elevated liver enzymes, bilirubin above the normal value or the onset of veno-occlusive disease (VOD) from the time of conditioning start and within the first 100 post-transplant days were considered as indicators for liver toxicity. The median stiffness of the liver measured by FibroScan on the day before conditioning treatment start was 7.6 kPa (range: 4.4–39.7). Six patients (15%) had prior liver diseases such as cirrhosis ( $n=1$ ), viral hepatitis ( $n=3$ ), steatosis ( $n=1$ ), or VOD ( $n=1$ ). The median onset of liver toxicity was day 0 (range: -2 until +92). The median bilirubin level of all 39 patients was 4 mg/dl (range: 0–17). The median AP level was 153 U/l (range: 80–833), the median GGT level was 343 U/l (range: 88–1647), the median ALT level was 108 U/l (range: 25–4481) and the median AST level was 67 U/l (range: 20–12292). The Pearson-test revealed a positive correlation between liver stiffness and the elevation of the AP ( $r=0.55$ ,  $P=0.001$ ) and GGT levels ( $r=0.54$ ,  $P=0.008$ ). The comparison of the median maximum enzyme and bilirubin levels is shown in Table 1. In two patients who developed severe VOD requiring defibrotide, the liver stiffness level was 6.9 kPa and 13.8 kPa, respectively. The patient with the highest stiffness level (39.7 kPa) developed acute GvHD of the liver, which completely resolved after steroid treatment. Only one of those five patients who had stiffness levels > 13 kPa died due to liver toxicity and concurrent septic shock, he suffered from viral hepatitis prior to transplantation.

Liver stiffness measured by transient elastography (FibroScan) positively correlates with the elevation of the cholestatic enzymes AP and GGT in myelofibrosis patients after allo-HSCT and may predict liver toxicity.

**Disclosure of conflict of interest:** None.

[P574]

Parameter	Median (range)/number	Median (range)/number	Significance
Liver stiffness (kPa)	≤ 7.6 (n= 20)	> 7.6 (n= 19)	
Bilirubin (mg/dl)	4.6 (0.6-15.3)	4 (0.8-17.4)	p= 0.945
ALT (U/l)	116 (28-4481)	108 (25-332)	p= 0.513
AST (U/l)	74 (89-12292)	63 (24-2503)	p= 0.627
AP (U/l)	133 (80-543)	237 (96-833)	p= 0.028
GGT (U/l)	339 (88-1052)	343 (133-1647)	p= 0.428
VOD	n= 1	n= 1	
aGvHD (liver)		n= 1	

## P575

### Impact of pre-transplant ruxolitinib in myelofibrosis patients on outcome after allogeneic stem cell transplantation

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Ruxolitinib (Rux) is the first approved drug for treatment of myelofibrosis. Because spleen size and constitutional symptoms may influence outcome after allogeneic stem cell transplantation (ASCT), Rux is recommended before stem cell transplantation in order to reduce therapy-related morbidity and mortality and improve outcome (EBMT/ELN recommendation, Leukemia 2015). The aim of this retrospective study was to evaluate the impact of pretreatment with Rux in comparison to transplantation of Rux-naïve MF patients with regard to outcome after ASCT. We included 149 myelofibrosis patients (pts) with a median age of 59 years (range: 28–74) who received ASCT between 2000 and 2015 from related ( $n=23$ ), matched ( $n=86$ ) or mismatched ( $n=50$ ) unrelated donor. All patients received busulfan-based reduced intensity conditioning. While 113 pts (66%) did not receive Rux, 46 pts (34%) received Rux at any time point prior to ASCT. The median daily dose of Rux was 30 mg (range: 10–40 mg) and the median duration of treatment was 28 days (range: 12–159 days). In 11 pts Rux was stopped before stem cell transplantation because of no response or loss of response, while in 35 pts Rux was given until start of conditioning. GvHD prophylaxis consisted of CNI plus short course MTX or MMF and anti-lymphocyte globulin. According to dynamic IPSS (DIPSS) ( $n=170$ ) the patients were either low ( $n=2$ ), intermediate-1 ( $n=36$ ), intermediate-2 ( $n=72$ ), or high risk ( $n=36$ ). As the median follow up was shorter for patients treated with Rux (15 vs 73 months,  $P < 0.001$ ). Primary graft failure was seen in 2 pts in the Rux and three in the non-Rux group. The median leukocyte engraftment was 13 days (range: 9–32) in the ruxolitinib and 14 days (range: 7–34) in the non Rux group ( $P=0.7$ ). The incidence of acute GvHD grade I to IV was significantly lower in the Rux group (49% vs 64%,  $P=0.05$ ), while aGvHD grade II-IV (33% vs 44%,  $P=0.14$ ) and grade III/IV (23% vs 25%,  $P=0.48$ ), did not differ significantly. The CI of NRM at 1 year was 18% (95% CI: 6–30%) for the Rux group and 22% (95% CI: 14–30%) for the non-Rux group ( $P=0.58$ ), and the CI of relapse at 2 years was 8% (95% CI: 0–16%) vs 20% (95% CI: 12–28%,  $P=0.25$ ). The 2 years RFS and OS was 66% (95% CI: 50–82%) and 69% (95% CI: 51–87) for the Rux group and 59% (95% CI: 49–69%) and 70% (95% CI: 62–78%) for the non-Rux group ( $P=0.29$  and  $P=0.45$ , respectively). Within the Rux group ( $n=53$ ), 24 pts responded to Rux (more than 25% spleen size reduction), while 29 pts did not respond or lost response prior to stem cell transplantation. Here, no significant difference could be seen between the responding and non-responding group for NRM (19% vs 17%,  $P=0.69$ ), Relapse (4% vs 13%,  $P=0.62$ ), RFS (61% vs 72%,  $P=0.81$ ) and OS (63% vs 75%,  $P=0.89$ ). In a multivariate analysis including Rux treatment as variable there was a non-significant trend in favor for Rux pretreatment regarding NRM (HR 0.79; 95% CI: 0.38–1.66,  $P=0.54$ ), relapse (HR 0.48; 95% CI: 0.18–1.31,  $P=0.15$ ), RFS (HR 0.55; 95% CI: 0.29–1.03,  $P=0.06$ ) and OS (HR 0.83; 95% CI: 0.41–1.67,  $P=0.61$ ). These results suggest that Rux pretreatment in myelofibrosis patient does not negatively influence outcome after allogeneic stem cell transplantation. To confirm the

observed favorable trend in outcome after Rux treatment more patients and a longer follow-up is needed.

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## P576

### Impact of tyrosine kinase inhibitor (TKI) towards allogeneic haemopoietic stem cell transplantation (allo-HSCT) among multi-ethnic chronic myeloid leukemia (CML) patients: A single Asian centre's experience

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In the tyrosine kinase inhibitor (TKI) era, allogeneic haemopoietic stem cell transplantation (allo-HSCT) has become the later-line therapy but still remains the only known curative treatment for chronic myeloid leukemia (CML). Since the introduction of TKI in our centre in 2004, the trend of allo-HSCT among our CML cohort has changed over time. The purpose of this study is to examine HSCT outcomes of our CML cohort who was either TKI naïve or has received TKI therapy prior HSCT. Between May 1999 and December 2015, 98 CML patients in our center received allo-HSCT with 39% were TKI naïve. The time of diagnosis to transplant was significant shorter among the TKI naïve group as compared to those received TKI prior HSCT (17.29 ± 7.29 months versus 42.33 ± 31.92 months, respectively). There were no gender different (60% males) but the median age at HSCT was younger among TKI naïve group, 29.50 years (range: 14–44 years) versus 33.50 years (range: 16–59 years) respectively. Malays remained majority ethnic group but the percentage was reduced among patients received TKI prior HSCT (60.5% versus 46.7% respectively). The disease phase at HSCT was significant different whereby majority of TKI naïve group was in first chronic phase (CP1) (60.5%) as compare to patients with prior TKI exposure (35.0%). All the patients in the TKI naïve group received HLA-matched related siblings donor (MRD) with 81.6% marrow stem cell source whereas only 88.8% of patients who have prior TKI exposure received MRD with 93.3% were from peripheral blood stem cell (PBSC). All patients in the TKI naïve group but only 73.3% among patients who have prior TKI exposure received full myeloablative conditioning regimen. There was slower neutrophil and platelet engraftment (19.97 ± 4.50 days versus 15.02 ± 3.55 days and 20.03 ± 6.72 days versus 13.93 ± 4.70 days respectively) among TKI naïve group. At 30 June 2016, the 1-year overall survival (OS) of CML at all disease status was 50% in TKI naïve group versus 32% for patients who have prior TKI exposure and transplanted in more advance disease stage. In general, patients in CP1 have the best OS. There was higher incident of grade 2 to 4 acute graft-versus-host-disease (GVHD) among the TKI naïve group (48.6% versus 16.7%, respectively) likely due to intensity of conditioning regimen with no significant different in chronic GVHD incident. Similarly, there was higher relapse rate among TKI naïve patients (44.7% versus 16.7%, respectively) as upfront post transplant TKI was not routinely given to this group of patients in the past. Further multivariate analysis to ascertain predictors of transplant outcome among this cohort of patients included disease status, donor-recipient gender combination, ethnic difference will be presented. In conclusion, despite emergent of effective and potent next generation TKI, HSCT still has its role as curative modality for patients who failed TKI. As showed in our data, the transplant outcome is excellent for patients who remain in CP1 at the time of HSCT and it is important to identify patients earlier, before disease progression, especially young patients, in order to optimize transplantation outcomes.

**Disclosure of conflict of interest:** None.

**P577****Long-term outcome of allogeneic hematopoietic stem cell transplantation (HSCT) for chronic lymphocytic leukemia (CLL): 10-year follow-up of the GCLLSG CLL3X trial**

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The purpose of this analysis was to provide 10-year follow-up of the GCLLSG CLL3X trial which aimed at evaluating reduced-intensity conditioning (RIC) HSCT in patients with poor-risk CLL. The CLL3X trial included 100 patients (median age 53 (27–65) years), of whom 90 patients were allografted with blood stem cells from related (40%) or unrelated donors (60%) using fludarabine-alkylator-based RIC regimens. 24% had refractory CLL at HSCT, and 35% had a TP53 deletion and/or mutation. The 6-year follow-up of the trial including the observation that genetic risk factors such as TP53 lesions and SF3B1 and NOTCH1 mutations had no prognostic impact has been previously reported. Survival and relapse information was requested for all patients who underwent HSCT within the CLL3X trial in 9 German centres (the Canadian centre was unavailable for follow-up) and were alive at the 6-year follow-up. RESULTS: Follow-up information was received for 37/44 patients (84%) alive at the 6-year follow-up. Of these, 5 patients had died (3 CLL,<sup>1</sup> chronic GVHD,<sup>1</sup> secondary cancer), and 3 had experienced disease recurrence. With a median follow-up of survivors of 9.7 (0.6–15.2) years, 10-year NRM, relapse incidence (REL), event-free survival (EFS), and overall survival (OS) of all 90 patients allografted was 25%, 55%, 31% and 51%, respectively, without significant effects of TP53 lesions on outcome. Absence of minimal residual disease (MRD) at the 12-month landmark post HSCT was highly predictive for a reduced relapse risk, in particular if MRD eradication occurred only after immunosuppression withdrawal, suggesting of effective graft-versus-leukemia activity (GVL; 10-year REL 12%). In the 32 patients who were alive and event-free 6 years post alloHCT, NRM, REL, EFS, and OS 4 years after this landmark (or 10 years after transplant) was 3.4%, 18%, 79%, and 94% with a median follow-up of 4.3 years (1.2–9.2) after the 6-year landmark. Notably, no relapse event occurred beyond 10 years post HSCT. Of those who remained event-free beyond 10 years, all 8 patients who were available for MRD assessment at their most recent follow-up were MRD-negative. Altogether 39 of the 90 allografted patients had CLL recurrence after transplant; 34 between 2003 and 2010, and 5 from 2011 onwards. Whilst the median survival of those patients who relapsed during the earlier period was 19 months, all 5 patients with late relapse are currently alive 4–62 (median 28) months after the event. CONCLUSIONS: Long-term observation of patients allografted in the CLL3X trial confirms that RIC HSCT can provide GVL-mediated sustained disease control in a sizable proportion of patients with poor-risk CLL independent of the TP53 status. Patients who are in MRD-negative remission one year after HSCT have an 87% probability of remaining disease-free at least for 10 years. However, late relapses do occur but may benefit from strategies involving innovative pathway inhibitors.

**Reference**

1. *Blood* 2013; **119**: 4851

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honoraria travel grants and research funding from AbbVie, Boehringer Ingelheim, Amgen, Celgene, Genentech, Genzyme, Gilead, GSK, Janssen, Mundipharma, Novartis, Pharmacyclics, Hoffmann-La Roche, Sanofi; S. Böttcher: Honoraria and research funding from AbbVie; honoraria, travel grants and research funding from Hoffmann-LaRoche; research funding from Celgene; U. Hegebart: Honoraria and financial support of conference participation from Jansen Cilag; M. Hallek: Consultancy and speakers bureau for Pharmacyclics, LLC and an AbbVie Company; speakers bureau for Janssen; M. Kneba: Consultancy, honoraria, travel grants and research funding from Gilead and Roche; consultancy, honoraria and travel grants from AbbVie and Janssen-Cilag; research funding from Amgen; travel grants from Glaxo-SmithKline; P.Dreger: Consultancy for Roche and Janssen; consultancy and speakers bureau for Novartis and Gilead.

**P578****No evidence for an increased GVHD risk associated with post-transplant Idelalisib given for relapse of chronic lymphocytic leukemia or lymphoma: First results of a survey by the EBMT chronic malignancy and lymphoma working parties**

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Idelalisib is a kinase inhibitor (KI) approved for the treatment of CLL and follicular lymphoma (FL). Idelalisib has a specific adverse effect profile including immune-mediated inflammatory conditions such as colitis and pneumonitis, raising concern about the safety of this KI if administered for treatment of malignancy recurrence after allogeneic hematopoietic cell transplantation (alloHCT). The purpose of this ongoing study is to provide information on the safety and efficacy of idelalisib in this setting. We included in this study adult patients who had been registered with the EBMT for an alloHCT for CLL or lymphoma and who received idelalisib for treating disease relapse or persistence at any time after transplant as indicated by participating investigators upon request by the EBMT study office in Leiden. Baseline patient, disease, and transplant data were collected from MED-A forms. Centers were requested to provide additional treatment and follow-up information. As of November 29, 2016, a total of 19 patients have been registered, of whom a full dataset as required for this analysis was available for 14 patients (CLL 9, FL 2, diffuse large B-cell lymphoma (DLBCL) 1, peripheral T-cell lymphoma 1, unspecified 1) who had undergone alloHCT between July 2009 and April 2015. All patients except one were male. Median age at transplantation was 52 (36–63) years and the median interval from diagnosis to alloHCT was 3.5 (0.8–12.2) years. Prior to alloHCT, 3 patients (1 CLL and 2 lymphoma) had received an autoHCT and two other patients had been exposed to KI (idelalisib 1, ibrutinib 1). Disease status at alloHCT was sensitive in 71% of the patients. Conditioning was reduced-intensity in 71% of the transplants and included *in vivo* T cell depletion in the majority of cases (71%). Donors were identical siblings in 43% with PBSC being the stem cell source in all cases. The interval between HCT and idelalisib commencement was 18 (2–68) months in the CLL group but only 3 (1–57) months in the lymphoma group. Prior to idelalisib, grade II–IV acute GVHD and chronic GVHD had been

observed in 7% and 36% of the patients, but was still active at the time of idelalisib commencement in only two cases (14%). Four patients with CLL had already failed ibrutinib given for post-HCT relapse prior to idelalisib. The median time on idelalisib until documented withdrawal or event (progression, retreatment, death) was 237 (9–569) days. After start of idelalisib, one patient developed grade 2 acute GVHD and subsequently chronic GVHD, however, in this patient idelalisib was started as early as 30 days after transplant. Efficacy of idelalisib in this high-risk patient sample was limited with only one PR in the CLL group (stable disease 4, progressive disease 1, not available 3; lymphoma not available), translating into a median event-free survival after start of idelalisib of 240 days. Five patients with CLL underwent a subsequent treatment with an alternate KI (ibrutinib 3, venetoclax 2). Altogether, there were five deaths, all due to disease progression (CLL 2, lymphoma 3). Median overall survival was 360 days for the whole sample and not reached for CLL. This preliminary data does not support concerns about the safety of idelalisib in the post alloHCT setting. Updated results of this ongoing study will be presented at the meeting.

**Disclosure of conflict of interest:** PD: Gilead: Speaker's bureau, Advisory Board.

#### P579

### Outcomes of patients with advanced CML treated with allogeneic hematopoietic stem cell transplantation in the Era of tyrosine kinase inhibitors (TKIs)

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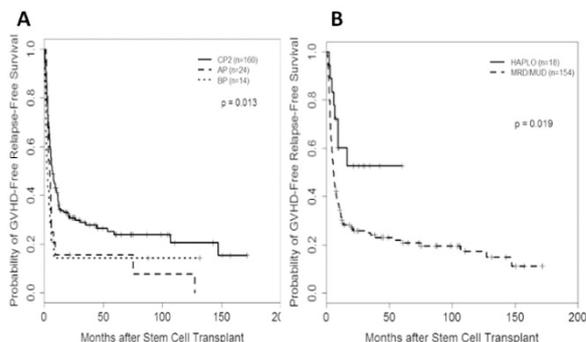
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Allogeneic hematopoietic stem cell transplantation (AHST) continues to be a potentially curative option for patients with advanced disease of who fail TKI therapy. Here we analyzed outcomes of patients with advanced CML (beyond first chronic phase—CP1) who received an AHST to identify factors associated with improved survival. A total of 207 consecutive patients with advanced CML treated at MD Anderson Cancer Center after year 2000 were included. The median age was 44 years (range: 2–70 years). Disease status at the time of transplant was second chronic phase (CP2), accelerated phase (AP) and blast phase (BP) in 160 (77%), 24 (12%) and 14 (7%) patients, respectively, and 9 (4%) patients had missing data. Persistent Ph-chromosome was detected in 129 (65%) patients, and 176 patients (85%) were less than a MMoIR at transplant. Forty of 114 tested patients (35%) had resistant BCR-ABL mutations and 10 patients (8.7%) had T315I mutation at transplant. Conditioning regimen was MAC in 140 patients (68%). Donors were matched related (MRD), matched unrelated (MUD), haploidentical, mismatched unrelated (MMUD), UCB and 1Ag mismatched related (MMRD) in 79 (38%), 75 (36%), 18 (9%), 17 (8%), 11 (5%) and 7 (3%) patients, respectively. The median follow-up duration of patients who survived at last follow-up was 60 months. At 30 days post-transplant, 180 of 200 tested patients (90%) and 134 of 201 tested patients (67%) achieved a CCyR and at least a MMoIR, respectively. The response to transplant by day 30 assessment correlated significantly with the disease status before transplant. A higher percentage of patients who experienced cytogenetic response before transplant experienced molecular response post-transplant (77%) compared with those who did not (61%;  $P=0.027$ ). For the entire group, the 1-year cumulative incidence (CI) of acute GVHD grade II–IV and grade III–IV were 41% and 15%, respectively; 5-year CI of extensive chronic GVHD was 31%. There was no significant

difference in the CI of severe acute or chronic GVHD between donor types. The CI of NRM at 100 days and 1 year was 14% and 30%, respectively. The CI of cytogenetic and molecular relapse at 5 years was 22% and 31%, respectively. Overall the 5-year OS, PFS and GVHD-free, relapse-free survival (GRFS) were 49%, 34%, and 22%, respectively. In multivariable analysis for GRFS, transplant in CP2 and the use of haploidentical donor significantly associated with better GRFS. The 5-year GRFS of patients in CP2, AP and BP before transplant was 24%, 16% and 14%, respectively ( $P=0.013$ ). (Figure 1A) Patients receiving a haploidentical donor had a better 5-year GRFS when compared with HLA matched transplants (53% vs 21%,  $P=0.019$ ). (Figure 1B) For PFS, transplantation in CP2, using a haploidentical donor and MAC regimen associated with better PFS while age, cytogenetic and molecular response before transplant did not predict survival. AHST is curative for a proportion of patients with advanced CML. PFS and GRFS are favorably influenced by percentage of BM blasts and donor type, with haploidentical donor having at least as good outcomes as HLA matched donors, while molecular and cytogenetic response before transplant do not appear to correlate with survival post-transplant

[P579]

Figure 1 GRFS based on percentage of BM blasts before transplant and donor types



**Disclosure of conflict of interest:** None.

#### P580

### Reduced-intensity conditioning with allogeneic stem cell transplantation in 25 patients with high-risk chronic lymphocytic leukemia: Single centre experience

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Allogeneic stem cell transplantation (SCT) has been considered as the treatment of choice for younger patients (pts) with high-risk chronic lymphocytic leukemia (CLL). Role of allogeneic SCT in era of novel drugs is widely discussed. Here we present our results after sequential use of chemotherapy and reduced-intensity conditioning (RIC) in cohort of 25 high-risk CLL pts. High-risk CLL was defined by one of the following: disease refractory to purine analogs, short response or early relapse (within 24 months) after purine analog combination treatment, and/or progressive disease with unfavorable genetic abnormalities (del [17p]/TP53 mutation). We analyzed 25 pts with high-risk CLL undergoing chemotherapy and RIC SCT in our centre from August 2007 to June 2016. The median of pretransplant lines were 3 (range: 2–4), novel drugs (idelalisib, ibrutinib) were used in 20% of pts (5/25). Fludarabine (30 mg/m<sup>2</sup>) and cytarabine (2 g/m<sup>2</sup>) for 4 days (FC) were used for cytoreduction in all pts. After 3 days of rest, RIC consisting of 4 Gy TBI, anti-thymocyte globulin 10–20 mg/kg/day for 3 days, and cyclophosphamide 40–60 mg/kg/day for 2 days followed. Median age of pts was 53 years (range:

32–62). Types of donors and used grafts were as follows: HLA identical sibling,  $n=6$ ; unrelated donor,  $n=19$ , PBSCs,  $n=23$ , BM,  $n=2$ . Median age of pts was 50 years (range: 38–61). Quantitative monitoring of minimal residual disease (MRD) after SCT was performed by four-colour flow cytometry and/or real time PCR. The median time of neutrophil engraftment (above  $0.5 \times 10^9/L$ ) was 16 days, 96% of pts (24/25) engrafted, one patient died in aplasia. Non-relapse mortality (NRM) after 1 year and 2 years was 8% (2/25) and 13% (3/25). Causes of death were refractory GVHD ( $n=1$ ), infection ( $n=1$ ) and multiorgan failure ( $n=1$ ). Incidence of acute GVHD was evaluated in 23 pts: 52% (12/23) of pts had GVHD (grade I-II in 7 pts, grade III in 5 pts). Incidence of chronic GVHD was evaluated in 22 pts, 41% (9/22) of pts had GVHD (limited in 6 pts, extensive in 3 pts). Treatment response was evaluated in 22 pts: complete remission was achieved in 19 pts (86%), MRD negativity after SCT was observed in 14 pts (64%). Donor lymphocyte infusions (DLI) were given to 7 pts (28%). Complete chimerism was achieved in 82% of pts in median of 65 days after SCT. With median follow-up from SCT 37 months (range: 4–90), 64% of all pts (16/25) were alive (14 in remission of CLL with MRD negativity, 2 with relapse), 9 pts died (3 from NRM, 6 from CLL relapse/progression), 8 relapses (32%; 8/25) occurred. Sequential use of chemotherapy and RIC regimen with allogeneic SCT is safety and effective treatment of high-risk CLL with response rate 86% and low NRM. Progression-free survival and overall survival at 3 years from SCT were 56% and 64%, respectively.

**Disclosure of conflict of interest:** None.

#### P581

##### **Role of allo-HSCT in the treatment of patients with T315I mutation in the TKI era**

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Treatment of CML is based on the use of tyrosine kinase inhibitors (TKIs). Despite the high effectiveness of the TKI, some patients in CP and significantly more patients in the AP and BC are resistant to it. The most important of the discussed mechanisms of resistance to TKI - emergence point mutations in the kinase domain ABL-tyrosine kinase. At present T315I mutation causing leukemia cells resistant to all known TKI I and II generation. Presents the results of 18 allogeneic bone marrow transplantation (allo-HSCT), made to 16 patients CML with T315I mutation and results data of pharmacological therapy of 37 patients provided hematological centers of the Russian Federation. At the time of allo-HSCT 4 patients were in CP1, in CP $\geq 2$ -7 patients, in AP-5, in BC-2 patients. In 7 cases donors were HLA—identical siblings, 11-unrelated donors. Fourteen patients were male, 2—woman. Age was 17–55 years (median—34 years). Therapy before allo-HSCT: 1 line therapy—two patients,  $\geq 2$  lines of therapy—three patients,  $\geq 3$  treatment lines—11. Time from diagnosis to SCT (months): 14–139, median—39 months. Time from detection of mutation to allo-HSCT (months) – 2–38, median—10 months. The number of points on EBMT scale: 3–4 points—12 patients, 5–7 points – 4 patients. Conditioning mode: 5 cases—MAC, 13—RIC. 7 patients out of 16 survived. At the time of SCT 2 patients were in the CP1, 4—in CP2, 1 AP. At present time all patient are in complete molecular remission: three patients in the 1st and 4—in remission achieved after the prophylactic administration of the TKI. The duration of observation of the living patients is far 8–79 months, median 48 months. OS patients with allo-HSCT—42.9%, with median of follow-up 18 months. Of the patients receiving pharmacological therapy survived 18 patients from 37. Male -25 patients, 12-women. Age was 17–77 years (median -49 years). At the time of diagnosis of the disease, 29 patients were in the CP, 8—in the AP. At the time of detection mutation 23 patients were in CP, 11- in AP, 3 - in

BC. Prior to the detection of mutation five patients received 1 line TKI therapy, 22–2 line TKI, 10–3 line TKI. After identifying the mutation was conducted therapy: ponatinib-3, I-II generation TKI-8, ChT+TKI/gidroxycarbamide+IF-17. At present time six patients are in CP $\geq 1$ , 9 patients—CHR, CCR \ PCR. The duration of observation of the living patient is far 53–250 months, median 81 months. OS patients, receiving pharmacological therapy – 48.4%, with a median follow-up 81 month. It is important not only to detect T315I mutation, but the phase of the disease in which it is found. Patient in CP1 may continue therapy TKI. Allo-HSCT is a potential therapeutic option for patients CML in the AP and BC with T315I mutation, especially for patients in CP $\geq 2$ .

**Disclosure of conflict of interest:** None.

#### P582

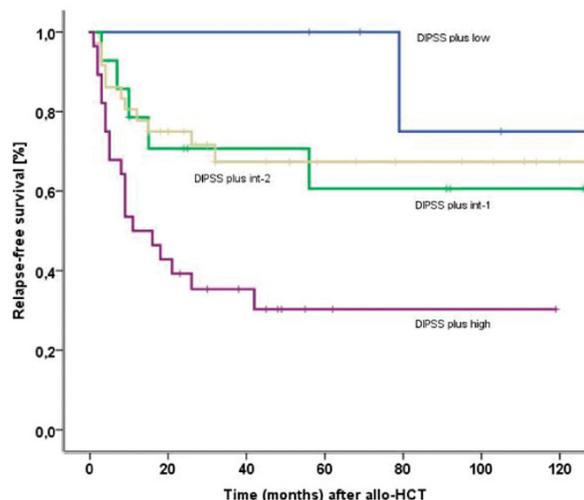
##### **Splenomegaly, high DIPSS plus score, ruxolitinib and chronic GvHD independently influence survival after myeloablative allo-HCT in patients with primary myelofibrosis**

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Primary myelofibrosis (PMF) is a chronic myeloproliferative stem cell disorder which is curable exclusively by allogeneic hematopoietic cell transplantation (HCT). 88 patients (pts) with PMF (53 male, 35 female; median age at HCT= 53 years) received HCT after myeloablative conditioning with total body irradiation (TBI) ( $n=28$ ), Treosulfan/ Fludarabine ( $n=19$ ), Busulfan/ Fludarabine ( $n=7$ ) or BCNU/ Melphalan/ Fludarabine ( $n=34$ ). Donors were HLA-identical ( $n=22$ ) or mismatched ( $n=3$ ) siblings and matched ( $n=48$ ) or mismatched ( $n=15$ ) unrelated. Transplants consisted of peripheral blood progenitor cells ( $n=85$ ) or bone marrow ( $n=3$ ). GvHD-prophylaxis was performed with CSA + MTX ( $n=37$ ), anti-thymocyte-globulin (ATG) ( $n=43$ ) or alemtuzumab ( $n=8$ ). Dynamic international prognostic scoring system (DIPSS) and DIPSS plus scores at time of HCT were generated where allocatable. Median follow-up (FU) for all pts was 30 months (56 months for survivors and 10 months for non-survivors), 1-year treatment-related mortality (TRM) was 32%. Primary graft-failure occurred in 4 pts (5%) and relapse was observed in 7 pts (8%) resulting in a 5-year (5-year) cumulative incidence (CI) for relapse of 5.76%. Five-year overall (OS) and relapse-free survival (RFS) was 59% and 57%. CI for cGvHD in pts surviving more than 3 months post-HCT was 35% after 5 years and 49% after 10 years. In a multivariate cox-regression model the occurrence of cGvHD independently improved OS ( $P=0.001$ , HR 0.27; 95% CI 0.12–0.59%) as well as RFS ( $P < 0.001$ , HR 0.19; 95% CI 0.08–0.46). High risk DIPSS plus score demonstrated significant inferior survival compared to intermediate-2 (OS  $P=0.006$ ; RFS  $P=0.009$ ), int-1 (OS  $P=0.037$ ; RFS  $P=0.042$ ) and low risk (OS  $P=0.021$ ; RFS  $P=0.014$ ) which could be confirmed in multivariate analysis for OS ( $P=0.001$ , HR 3.13; 95% CI 1.56–6.3) and RFS ( $P < 0.001$ , HR 4.84; 95% CI 2.05–11.43). RFS additionally was improved by splenomegaly ( $n=60$ ) vs. normal spleen size ( $n=11$ ) at time of HCT ( $P=0.01$ , HR 0.29; 95% CI 0.1–0.75). Ruxolitinib ( $n=20$ ) or none ( $n=45$ ) pre-treatment compared to other drug therapy ( $n=19$ ) resulted in improved OS ( $P=0.013$ ) and RFS ( $P=0.031$ ) and was an independent factor for RFS in multivariate analysis ( $P=0.046$ , HR 0.39; 95% CI 0.16–0.99). Non-relapse mortality (NMR) was significantly determined by high-risk DIPSS plus score ( $P=0.001$ ) or DIPSS high and int-2 ( $P=0.009$ ). Relapse incidence was significantly lower in pts with splenomegaly compared to asplenic or normal-spleenized pts prior to HCT ( $P=0.027$ ). Our data point out that pre-therapy and DIPSS or DIPSS plus score are relevant pre-transplant outcome factors while chronic GvHD is the most important independent HCT-related factor. Furthermore, splenomegaly at HCT reduces risk of relapse and therefore improves RFS.

[P582]



**Disclosure of conflict of interest:** None.

**P583**

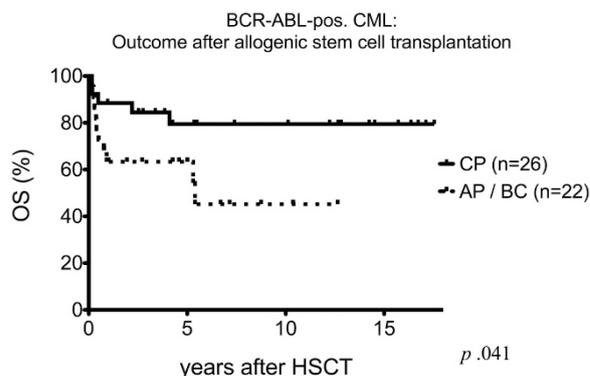
**The role of allogeneic hematopoietic stem cell transplantation (HSCT) for chronic myeloid leukemia (CML) patients in the TKI-era: A single center experience**

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In the era of tyrosine kinase inhibitors (TKI) as superior first line treatment in the therapy of CML, the concept of allogeneic HSCT has been pushed to the role of salvage therapy. To date, data on allogeneic HSCT after TKI-therapy are scarcely available. In this study, we report single center data on the outcome of 52 CML patients, for the most part pretreated with TKI, who underwent allogeneic HSCT between 1999 and 2015 with a follow-up of 12 months to 17 years. Upon obtaining written informed consent 48 patients diagnosed with BCR-ABL-positive CML and 4 patients with BCR-ABL-negative atypical CML were included in this analysis. The majority of patients underwent myeloablative conditioning regimen. The median age at time of HSCT was 47 years with a range: from 19 to 67 years. Twenty-one patients were transplanted from a matched related donor, and 31 received stem cell grafts from an unrelated HLA-compatible donor. 36/48 patients received TKI-therapy before transplantation, 23 patients received more than 1 TKI prior to HSCT. 10/48 patients were treated with interferon prior to HSCT. Twenty-two patients were transplanted due to acceleration or blast crisis. Twenty-six patients received an allogeneic HSCT in chronic phase (CP, n=16) or complete hematologic (CHR, n=7) or cytogenetic remission (CCyR, n=3). Kinase domain mutations could be identified in seven patients including T315I-mutation in four patients. Seven patients showed "major route" cytogenetic aberrations. Next to advanced disease status, TKI intolerance (n=4) and TKI resistance (n=11) were the main indications for HSCT after 2001. After a median follow up of 5 years and 3 months, those 26 patients transplanted in CP, CHR or CCyR showed an overall survival (OS) of 79%. 3/27 patients died in remission and two patients died after CML relapse. After 2004 none of the 15 patients transplanted in CP, CHR or CCyR died or relapsed so far, with a median follow-up of 1672 days. All of these patients received TKI therapy prior to transplant. Twenty-two patients transplanted in advanced stage CML (BC and AP) had after a median follow up of 5 years an OS of 44%. The difference between survival curves is significant (Log rank test P=0.041;

HR 0.3407, 95% CI of ratio 0.1211–0.9585). Prior to transplantation 19 of these patients received a TKI-therapy. In this group, four patients died due to CML relapse, one died after development of donor cell leukemia and five patients died in remission. One patient with atypical CML was transplanted in BC and died of progressive disease shortly after transplantation. The other three patients with atypical CML were transplanted in CP-phase. With a median follow-up of 648 days these patients are in ongoing remission. Even in times of TKI-therapy allogeneic HSCT remains a successful and safe therapy option for CML patients with TKI intolerance or resistance. Patients transplanted in CP or complete remission had an excellent long-term outcome. Allogeneic HSCT should be considered in TKI resistance or intolerance before the development of blast crisis. Despite TKI therapy, overall survival deteriorates in patients with advanced disease. However, this treatment modality can improve survival rates substantially compared to other available therapies. TKI-maintenance therapy could be a possible strategy to prevent CML relapse, although randomized data on TKI-maintenance therapy after allogeneic HSCT are still lacking.

[P583]



**Disclosure of conflict of interest:** None.

**P584**

**Use of first or second generation TKI for CML after allogeneic hematopoietic stem cell transplantation: A study by the CMWP of the EBMT**

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Patients (pts) relapsing with CML after allogeneic hematopoietic stem cell transplantation (alloHSCT) may be treated with TKI and/or DLI. As nowadays the majority of CML pts would have received at least imatinib prior to transplantation, we were interested in analyzing (a) the type of TKI used after alloHSCT, (b) the indication for TKI treatment, (c) the outcome of this treatment and d) the temporal relationship with DLI if given. 435 pts received TKI after first allogeneic HSCT for CML. Transplants had been performed in CP1,  $n=194$ , AP,  $n=60$  or for more advanced disease (BC/> CP1,  $n=177$ ) from HLA identical siblings ( $n=231$ ) or UD ( $n=204$ ) between 2000 and 2013. TKI given prior to transplant was imatinib ( $n=268$ ), dasatinib ( $n=162$ ), nilotinib ( $n=88$ ), bosutinib ( $n=4$ ) and ponatinib ( $n=7$ ). Median age at transplant was 44 (18.5–68) years, 274 pts (63%) were male. TKI post alloHSCT were given between 2000 and 2015. First TKI given was either imatinib ( $n=223$ ), dasatinib ( $n=131$ ), nilotinib ( $n=67$ ), bosutinib ( $n=2$ ) or ponatinib (12). The indications for TKI therapy were the same as for transplantation ( $n=25$ ), for relapse/progression/persistent disease ( $n=246$ ), for prophylaxis/pre-emptive ( $n=147$ ), planned ( $n=5$ ), others ( $n=8$ ) and missing ( $n=4$ ). Median follow-up from start of TKI was 55 (1–171) months. The median time interval from transplant to TKI was 6 (0.2–165) months. It was longer for TKI given for relapse/progression with 15 (1–89) months and shorter for TKI given for

prophylaxis/pre-emptive with 1.6 (0.2–43) months. It was longer for imatinib with 11 (0.2–121) months vs 3.8 (0.2–165) months for other TKI. 103/223 (46%) of pts with imatinib, 99/131 (76%) with dasatinib, 55/67 (82%) with nilotinib and 11/14 (79%) with bosutinib/ponatinib post-transplantation had been treated with imatinib prior to transplantation. In total, 196 (45%) patients received DLI after alloHSCT, of which 63/435 (14.5%) had DLI prior to TKI post-alloHSCT, 19/435 (4.4%) had DLI at the same time of TKI and 114/435 (26%) had DLI post-TKI. Best response after TKI was complete molecular remission in 17.7%, cytogenetic remission in 4.4%, hematological remission in 20.2% and no response/progression/relapse in 57.7% of pts. 50% of pts treated with imatinib had a response (molecular/cytogenetic/hematological) vs 34% with nilotinib, 33% with dasatinib and 33% with bosutinib/ponatinib,  $P=0.014$ . In univariate analysis, OS, RFS and RI were better for imatinib vs other TKI (Table 1). In multivariate analysis for OS, imatinib vs other TKI post-transplant did not show anymore an effect, HR 1.19 (0.85–1.67),  $P=0.317$ . Factors influencing OS were time from diagnosis to transplant, HR 1.01 (1.00–1.01),  $P=0.009$ , AP vs CP1, HR 1.80 (1.11–2.91),  $P=0.017$  and BC/>CP1 vs CP1, HR 2.3 (1.58–3.33),  $P<0.0001$ . In multivariate analysis for RFS as for OS, imatinib vs other TKI did not have an effect, HR 1.11 (0.83–1.48),  $P=0.496$ . Other factors having a tendency or influencing RFS were time from diagnosis to transplant, HR 1.00 (1.00–1.01),  $P=0.054$ , AP vs CP1, HR 1.52 (1.00–2.31),  $P=0.050$  and BC/>CP1 vs CP1, HR 2.11 (1.55–2.88),  $P<0.001$ . These data suggest that TKI after alloHSCT induce a response in about 42% of pts regardless of the type of TKI used and that time from diagnosis to transplantation as well as the phase of disease at transplant remain the main factors influencing the outcome of CML patients relapsing after alloHSCT.

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[P584]

	All patients	Imatinib	Other TKIs	p
5-yr OS	60% (55-65%)	66% (60-73%)	51% (42-60%)	0.0024
5-yr RFS	47% (42-53%)	53% (46-60%)	40% (32-48%)	0.0102
5-yr RI	25% (21-30%)	21% (16-27%)	31% (24-38%)	0.0454
5-yr NRM	27% (23-32%)	26% (20-31%)	29% (22-36%)	0.365

## Haemoglobinopathy and Inborn errors of metabolism

### P585

#### A treosulphan based protocol for thalassaemia major—graft kinetics in different age groups

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Thalassaemia major affects 10 000 new babies in India each year and haematopoietic stem cell transplantation offers the only chance of cure. We present data on 179 children with thalassaemia major aged between 9 months and 17 years using a uniform conditioning regimen consisting of thiotepa 8 mg/kg, treosulphan 42 gm/m<sup>2</sup> and fludarabine 160 mg/m<sup>2</sup>. Equine antithymocyte globulin at a dose of 45 mg/kg was added to children who were undergoing transplantation from an unrelated donor source. There were eight deaths before engraftment due to sepsis or bleeding and two related to graft versus host disease. All patients showed complete chimerism on day 28. However, in 21 children there was an acute drop in donor chimerism between day 60 and 120 post transplantation. Immunosuppression was abruptly stopped when donor chimerism dropped below 95% in all children. Seven children responded well and re-established complete chimerism with this measure. Seven children progressed to develop complete graft loss. Donor lymphocyte infusion (DLI) in the form of small aliquots of peripheral whole blood from the donor was administered in seven children. DLI was used in a graded fashion every 2 weeks starting from 1 × 10<sup>4</sup>/kg of CD3, followed by 1 × 10<sup>5</sup>/kg and 5 × 10<sup>5</sup>/kg. All of them continued to maintain their graft with these interventions. Drop in chimerism was seen particularly in children less than 3 years at the time of transplantation comprising 14 out of 21 children. Older children with Lucarelli Class III were also prone to rejection in our earlier series and this complication has now been eliminated with pre-transplant immunosuppression and hypertransfusion. Children above the age of 7 years were more prone to graft versus host disease and required on average 18 months of immunosuppression. Treosulphan based protocol has been equally well tolerated by all age groups, all Lucarelli classes of children with thalassaemia major and different donor sources. The transplant related mortality and graft rejection rates have been low at 5.5% and 3.9%, respectively. However, children less than 3 years need to be monitored carefully during the first 4 months of transplantation as early withdrawal of immunosuppression can prevent graft rejection resulting in excellent outcomes.

**Disclosure of conflict of interest:** None.

### P586

#### Allogeneic hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis (HLH) in adults: A retrospective study of the chronic malignancies and inborn errors working parties (CMWP and IEWP) of the EBMT

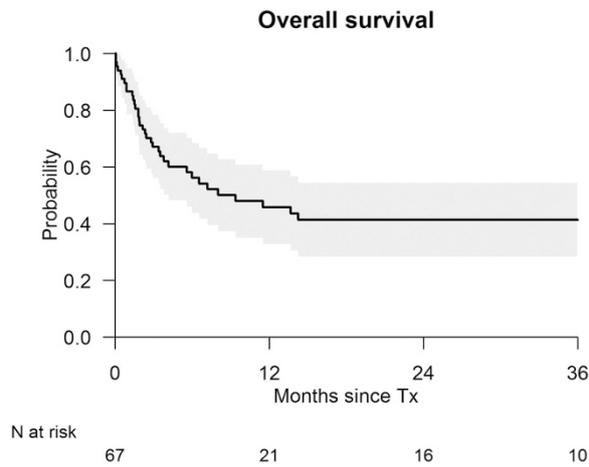
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Hemophagocytic lymphohistiocytosis (HLH; hemophagocytic syndrome) is a rare syndrome of potentially fatal, uncontrolled hyperinflammation. Allogeneic stem cell transplantation (alloSCT) is indicated in familial, recurrent or progressive HLH. Additional recommendations include central nervous system involvement and unknown triggering factor. While data for alloSCT outcome are available for the pediatric setting, information for adults is very limited. The aim of this study was to retrospectively analyze the information from the EBMT databases about adult HLH patients who underwent allogeneic stem cell transplantation. We obtained data of 67 adult (≥18 years of age) patients transplanted due to HLH. Additionally, an HLH-oriented questionnaire was sent to the clinical centers, with 23 responses received so far. Median age at transplantation was 28 (range: 18–65). There was a slight male predominance 43/67 (64%). The majority of patients were reported with secondary HLH 33/67 (49%), the familial disease was reported in 29/67 (43%) patients. In two patients triggering factor was attributed to malignancy. The majority of patients received stem cells obtained from the peripheral blood (52/67; 78%) while for the remaining ones it was bone marrow. Reduced intensity conditioning was used since 2004 in 23/63 (37%) of patients. Thirteen (19%) patients received TBI. Donor choice was: 33 matched unrelated (50%), 7 mismatched unrelated (11%), 26 identical sibling (39%). Engraftment was observed in 55/61 (77%). The cumulative incidence of acute graft versus host disease (GvHD) at 100 days was 26% (95% CI 15–37%). The cumulative incidence of chronic GvHD at 1 year after alloSCT was 13% (95% CI 2–23%) and increased to 31% at 3 years (95% CI 16–47%). The 3-year probability of overall survival is shown in Fig.1. The median survival time was 8 months. The 3-year OS was 41% (95% CI 28–55%). For patients who survived until 3 months, this proportion was more favorable with an OS of 62% (95% CI 45–78%) at 3 years after transplantation. Among 19 patients with observation times longer than 15 months, only one patient died (in the 48th month after alloSCT due to relapse which occurred in the 12th month. After 12 months no more relapses of HLH were recorded—the cumulative incidence reached 19%. The non-relapse mortality reached 42% after 15 months. The familial disease was associated with a better prognosis than secondary HLH ( $P=0.04$ ). Unlike the pediatric population, where reduced intensity conditioning (RIC) was associated with higher survival, in adult patients there was no difference between the conditioning types. Data from the 23 questionnaires confirm clinical picture typical for HLH at the diagnosis: fever in 21/22 (95%), splenomegaly in 19/20 (95%), hemophagocytosis in 18/20 (90%) and hyperferritinemia with median concentration of 4215 ng/ml (range: 63–260 160). Image Fig.1 Overall survival after allogeneic stem cell transplantation for adult HLH patients until 36 months (95% Confidence Intervals are shown in grey). The number of patients at risk is indicated below the time axis at the corresponding time points. To our knowledge, this is the largest group of adult patients with HLH who underwent allogeneic stem cell transplantation. Relatively low relapse incidence shows that alloSCT can effectively cure HLH. Patients who survive the first period after this procedure can expect a long disease-free survival.

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**P587**

**Allogeneic hematopoietic stem cell transplantation with post-transplantation cyclophosphamide for patients with inherited non-malignant diseases**

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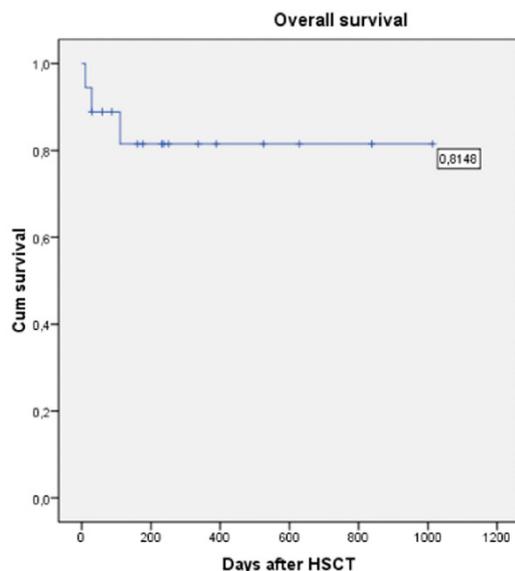
Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative option for children suffering from various life-threatening inherited non-malignant diseases with best results using HLA-identical family donor. HSCT from unrelated or mismatched family donors is associated with increased risk of aGVHD and graft rejection. Use of post-transplantation

cyclophosphamide (PTCY) with or without additional immunosuppression has been shown to be effective prophylaxis against GVHD in patients with hematological malignancies. There are limited reports of HSCT using PT CY for patients with non-malignant disorders. We retrospectively analyzed results of 18 HSCT in patients with life-threatening non-malignant diseases using PTCY-based GVHD prophylaxis. Patients characteristics are presented in Table 1. Thirteen patients (72.2%) were transplanted upfront, 5 patients (27.8%) were rescued after primary or secondary graft failure after first HSCT. Donors were HLA-matched ( $n=8$ ) or mismatched (8–9/10) ( $n=4$ ) unrelated, haploidentical ( $n=5$ ) or HLA-identical family ( $n=1$ ). Bone marrow was used as graft source in 13 (72.2%) patients and peripheral blood stem cell in 5 (27.8%). Median CD34+/kg recipient weight- $7.25 \times 10^6$  (3.0–13.5), CD3+/kg- $9.785 \times 10^7$  (0.78–90.6). The conditioning regimen was myeloablative in 12 patients (conventional-3, reduced toxicity-9), reduced intensity-6. The GVHD prophylaxis consisted of a combination of PTCY at dose of 50 mg/kg on Days +3 and +4 with calcineurin inhibitors (Tacrolimus-6 pts, Cyclosporine A-10 pts) or Sirolimus (1 pt) and MMF (15 pts) starting on day +5. All but one patients received also serotherapy with rabbit (12 pts) or horse ATG (5 pst) and Rituximab (4 pts). With a median follow-up of 8 months (range: 1–33), the Kaplan–Meier estimates of OS –81.5%. One patient with thalassemia died before engraftment on day+11 from severe VOD. 15/17 pts (88%) achieved engraftment. The median time for neutrophil and platelet engraftment was 23 (16–28) and 19 days (12–32), respectively. Primary graft failure was observed in 2 patients (1 was successfully retransplanted from another haploidenticle donor, 1 was not eligible for a second transplantation, but alive). At last follow up, 10 (67%) patients had full donor chimerism, 4 (27%) had stable mixed chimerism without signs of disease progression. One patient with Wiscott-Aldrich syndrome had secondary graft failure with progressive loss of donor chimerism and were successfully rescued with second haploidentical transplant from the same donor. Of 15 engrafted patients, aGVHD II-IV was seen in 4 (26.7%) patients. One patient developed grade II (gut stage II) aGVHD, which resolved with systemic steroids. Severe (grIII–IV) aGVHD was observed in 2 pts after second HSCT, both had calcineurin and mTOR-inhibitors induced toxicity leading to discontinuation of this drugs, but responded on combined (steroids and Ruxolitinib) therapy. One patient with WAS developed grade III GVHD (gut stage 4) after severe CMV-colitis and died on day from multiple organ failure (suspected TMA). One patient developed extensive chronic GVHD of kidney (minimal change

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**Table 1 Patients characteristics (n=18).**

Sex (M:F)	(2,6:1)
Age at diagnosis, months; Mediana (range)	16,5 (0-18)
Age at HSCT, years; Mediana (range)	2,5 (1-20)
Time from diagnosis to HSCT, months Mediana (range)	14,5 (4-18)
<b>Diagnosis</b>	
MPS1 (Hurler syndrome)	7 (38,8%)
Thalassemia	5 (27,8%)
Farber disease	1 (5,6%)
Wiscott-Aldrich syndrome	4 (22,2%)
X-linked adrenoleukodystrophy	1 (5,6%)



disease) after tapering of immunosuppression. One patient with Hurler syndrome had seizures, died on day+29 from multiple organ dysfunction syndrome. Conclusion: PTCY is a promising option for aGVHD prophylaxis in patient with non-malignant disease, lacking an HLA-matched family donor.

**Disclosure of conflict of interest:** None.

#### P588

##### **An exploratory, open-label study to evaluate the safety and feasibility of ATIR201, a T-lymphocyte enriched leukocyte preparation depleted *ex vivo* of host alloreactive T-cells (using photodynamic treatment), as adjuvant treatment to a T-cell depleted haploidentical hematopoietic stem cell transplantation in patients with beta-thalassemia major**

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Previous studies demonstrated that donor lymphocytes, selectively depleted of alloreactive T-cells (ATIR), could be given safely in adult patients receiving a haploidentical HSCT. In 42 patients a single dose of ATIR, at doses up to  $2 \times 10^6$  viable T-cells/kg, was given and no grade III/IV acute GVHD has been reported. This confirms the efficacy of the elimination method of allo-reactive T-cells and attributes to its beneficial safety profile. In an ongoing phase 2 study, CR- AIR-007 (NCT01794299), infusion of ATIR101 at 28 days post-HSCT results in a reduction of transplant-related mortality (TRM) and improvement of overall survival and event-free survival. Adjuvantive treatment with donor lymphocytes in patients receiving a T-cell depleted, haploidentical HSCT for non-malignant diseases such as beta thalassemia major, could provide early immunological support and better immune reconstitution in the absence of GVHD. In an open-label, multicenter phase 2 study (CR-BD-001; EudraCT 2016-002959-17), 10 patients age  $\geq 2$  years and  $\leq 25$  years with beta thalassemia major will undergo a haploidentical HSCT with adjunctive administration of ATIR201. Patients will receive a T-cell depleted graft (CD34-selected, or CD3/CD19 depleted, or TCR- $\alpha\beta$  depleted, depending on the experience of the study center) from a related, haploidentical donor, Patient conditioning will be myeloablative following standard practices at the study center. ATIR201 infusion at a dose of  $2 \times 10^6$  viable T-cells/kg is given between 28 and 32 days after the HSCT. To assess safety, patients will be evaluated for the occurrence of dose limiting toxicity (DLT), defined as acute GVHD grade III/IV within 180 days post HSCT. Efficacy will be primarily evaluated by transfusion-free survival (TFS), occurrence of severe infections, and time to T-cell reconstitution, taking into account hematologic and sustained engraftment. All patients will be closely monitored for CMV, EBV and Adenovirus titers, with initiation of pre-emptive treatment upon rising blood titers. Regulatory authorities in the United Kingdom and Germany have approved this clinical study protocol. Enrolment of the study is expected to continue during 2017, with first report of safety of ATIR201 to be expected first half 2018.

**Disclosure of conflict of interest:** J. Rovers is employee of Kiadis Pharma, sponsor of the study.

#### P589

##### **Changing the shape of sickle cell disease treatment: A hermeneutic study of a case that changed a family and a medical practice**

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Sickle cell disease (SCD) can be cured with haematopoietic cell transplantation (HCT), yet progress in the practice and research of HCT for SCD has not come without risks and uncertainty. The information and decisions that families and physicians encounter in this field are complex and hanging. In this hermeneutic study, we analyze the case of one family who advocated for HCTs for two of their four children knowing the potential risks. These experiences have had a profound impact on both the family and the medical team. This study was conducted through the research method of hermeneutic phenomenology. Hermeneutic inquiry is described as the practice and theory of interpretation and understanding in human contexts and aims to make sense of the particulars of these contexts and arrive at deeper understandings. Data Collection: In-depth interviews were conducted with the mother of the family, the HCT nurse coordinator, and the HCT physician. The interviews were audiotaped and transcribed verbatim. The transcribed interviews were later reviewed by the physician, who then wrote an additional reflection. This work culminated in approximately 70 pages of single spaced data in textual form. In hermeneutics, interpretation takes place through a careful reading and re-reading of the data, looking for statements and instances that resonate with the researcher. Initial individual interpretations of researchers are then raised to another level of interpretive analysis in the research team's communal attention to the data. Particular criteria guide the analysis: agreement, coherence, comprehensiveness, potential, and penetration. The following excerpts and interpretations are provided as examples of the analysis, with names changed for confidentiality. "Being heard" arose repeatedly in this family's experience, including at the time of their request for a transplant without meeting the traditional criteria for HCT. They persisted in their belief that their children would benefit from HCT. "They gave us options to see if there was a chance for a transplant...how life would look.... And then we figured...a transplant for him was better at the time...worth the risks.... And you wouldn't even know. He plays basketball now, he plays sports, he's active and he can exercise and run. I never had any regrets because I felt it was better and the most important thing is his organs were really intact; none of the organs were destroyed...so I think it's the right decision we made" (mother). "This family has changed my career, and my life as a result. They challenged my practice and way of thinking. They did so in a considerate way, out of a duty to advocate for their children. We worked through the tension of different viewpoints with respect and all of us grew in the process. At least I can say our team did. I certainly did... I am humbled by their trust and respect...I am grateful to them" (physician). Patients and providers are deeply impacted by their interactions. Dr. Robert Buckman stated that it was the individual case that changed his practice always. He claimed he could not walk into a new patient's room without his practice being forever changed. In presenting this hermeneutic analysis, we aim to remind ourselves of the opportunity for growth that can result from reflection on this sacred patient-provider relationship.

**Disclosure of conflict of interest:** None.

#### P590

##### **Defibrotide (DF) prophylaxis and adjustment of busulfan schedule to prevent veno-occlusive disease and thrombotic microangiopathy in an infant with a membrane cofactor protein (MCP) gene mutation and metachromatic leukodystrophy undergoing hematopoietic stem cell gene therapy (HSC-GT)**

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Hepatic veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA) are life-threatening complications that can occur after HSC transplantation. Expert consensus guidelines support use of DF for treatment and prophylaxis of VOD due to its ability to restore thrombo-fibrinolytic balance and protect endothelial cells. Presymptomatic monozygous twins affected by late infantile Metachromatic Leukodystrophy (MLD) underwent investigational HSC-GT after conditioning with busulfan. No comorbidities were evident at baseline. The dose of transduced CD34+ cells was similar in both patients ( $18.2 \times 10^6$  CD34+/kg for Patient 1 and  $14.1 \times 10^6$  CD34+/kg for Patient 2). Patient 1 (P1): at 8 months of age, received conditioning with iv busulfan 80 mg/m<sup>2</sup>/dose for 4 doses (target AUC 85 mg×h/L). On day (d) +18 after GT, he developed severe VOD and was treated with diuretics, fresh frozen plasma, paracetamol and DF. On d+39 schistocytes in peripheral blood, marked proteinuria, complement factor consumption, and increases LDH and bilirubin were observed. The patient's condition worsened, with reduced urine output and generalized oedema with pleural effusion. Stool, urine and blood cultures were negative and ADAMTS13 activity was 35%; anti-complement factor H (CFH) antibodies (ab) were positive (1371 UI/mL). These findings led to the diagnosis of atypical hemolytic uremic syndrome (aHUS; a form of TMA) and eculizumab (300 mg/weekly dose) was started on d +40. Patient subsequently developed pulmonary oedema and needed non-invasive ventilation. Molecular analysis revealed a heterozygous deletion of CFHR3-R1 and Ala353Val mutation in the MCP gene, a defect previously shown to be associated with aHUS. Due to the presence of ab anti-CFH and anti-platelet, 4 weekly doses of rituximab (375 mg/m<sup>2</sup>) were administered. After treatment, P1 progressively improved although he showed prolonged severe anaemia and thrombocytopenia and bone marrow (BM) hypoplasia, secondary bleeding which required reinfusion of unmanipulated autologous BM cells on d +66. Nine months after HSCT-GT P1 has shown good hematopoietic recovery, stable engraftment of the transduced HSCs, no signs of renal damage or complement activation, albeit with neurodevelopmental delay. Patient 2 (P2): given his twin history and genetics, this 9 month old infant was considered at increased risk of VOD, so prophylaxis with DF (25 mg/Kg/d) was administered from d-4 to d+30 and the busulfan conditioning was modified by adjusting the AUC to a lower target (1 mg/kg/dose for 14 doses; target AUC 67.2 mg × h/L). The child had a good clinical recovery and didn't develop signs of VOD or TMA after HSC-GT. On d+12 and +14, respectively, anti-CFH and anti-platelet ab were positive. Considering the history of the twin, 4 weekly doses of rituximab were administered. P2 is currently 8 months after GT with persistent engraftment of transduced HSCs and no signs of TMA. Data from this case-control report of monozygous twins diagnosed with MLD, and subsequently

shown to also harbor mutations in complement regulator gene, suggest that DF prophylaxis and busulfan adjustment may have helped prevent systemic microangiopathic damage in the second twin. Patients with rare disease may have mutations in genes in addition to those that cause their disease. Patients at risk of post-transplant TMA following HSC-GT for genetic diseases may require tailored DF prophylaxis and treatment.

**Disclosure of conflict of interest:** A. Aiuti is the principal investigator of the TIGET-MLD clinical trial of gene therapy. The MLD gene therapy was licensed to GlaxoSmithKline (GSK) in 2014 and GSK became the financial sponsor of the trial. All authors declare no other competing interests.

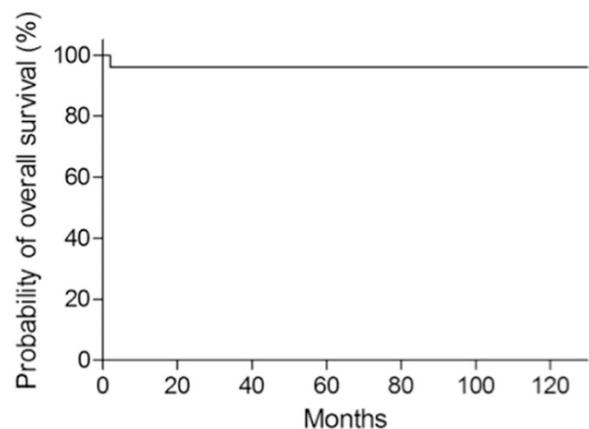
## P591

### Disease-specific conditioning regimen in hematopoietic stem cell transplantation for children with inherited bone marrow failure syndromes

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Hematopoietic stem cell transplantation (HSCT) using an optimized conditioning regimen is essential for the long-term survival of patients with inherited bone marrow failure syndromes (IBMFS). We report HSCT in 25 children with Fanconi anemia (FA, n=12), Diamond-Blackfan anemia (DBA, n=7), dyskeratosis congenita (DC, n=5) and Shwachman-Diamond syndrome (SDS, n=1) from a single HSCT center. The graft source was peripheral blood stem cells (n=20) or cord blood stem cells (n=5). FA, DC and SDS patients received reduced-intensity conditioning, while DBA patients had myeloablative conditioning. The median numbers of infused mononuclear cells and CD34+ cells were  $14.4 \times 10^8$ /kg and  $4.5 \times 10^6$ /kg, respectively. The median time for neutrophil and platelet recovery was 12 and 17 days, respectively. There was one primary graft failure. After median follow up 3 years the overall survival was 96%. The incidence of grade II-III acute and chronic graft versus host disease (GvHD) was 32% and 16% respectively. In a multivariate analysis, the type of conditioning regimen was the only factor identified as significantly associated with grade II-III acute GvHD (P=0.01). We conclude that HSCT can be a curative option for patients with IBMFS. Disease specific conditioning regimen was important to disease the transplant-related mortality.

[P591]



**Disclosure of conflict of interest:** None.

## P592

### Haploidentical hematopoietic bone marrow transplantation with consecutive living kidney transplantation from the same donor in a sickle cell disease patient with end-stage renal failure

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Homozygous Sickle cell disease (SCD) patients suffering from end-stage renal disease (ESRD) show a variable outcome after kidney transplantation as underlying disease can cause poor allograft survival and disease-specific problems. We present a case of a 27-year old patient with severe SCD and ESRD who underwent haploidentical bone marrow transplantation (BMT) with consecutive living kidney transplantation (LKT). The patient suffered from multiple complications of SCD including stroke with secondary hemorrhage, symptomatic epilepsy, ESRD and uncontrolled hypertension. The patient had been on hydroxyurea without success and required regular blood transfusion due to severe renal anemia. The rationale for BMT was uncontrollable iron overload. A reduced intensity conditioning regimen was used with (fludarabine, cyclophosphamide and 2Gy of TBI, dose-adjusted to ESRD). Graft-versus-host disease (GvHD) prophylaxis consisted of post-transplant high-dose cyclophosphamide, cyclosporine A (CyA) and mycophenolate mofetil (MMF). The donor was her 56-year old mother with HbS trait, the stem cell source was bone marrow, the cell dose  $4.74 \times 10^8$  nucleated cells/kg. During conditioning daily hemodialysis was performed to keep drug levels stable. Neutrophil engraftment occurred on day +26, chimerism at day +19 was 98%. HbS increased from 1.3% pre-HSCT to 40.0% 6 months after HSCT. Hemoglobin values increased from 70 g/L pre-HSCT to 110 g/L post-HSCT and reticulocytes from 16 G/L to 124 G/L. Erythropoietin levels increased from 2.3 IU/L pre-HSCT to 178 IU/L 6 months after HSCT. During the follow-up, the patient did not show any sign of acute GvHD or vaso-occlusive crisis, hemolysis or sickling. Relevant complications were disease-related (therapy resistant hypertension and epileptic seizure due to former brain damage). On day +151 a LKT from the same donor was performed. The initial immunosuppressive treatment with MMF was continued, CyA was switched to tacrolimus and steroids were added for 3 months. The post-transplant period was uneventful. Currently, 12 months after haploidentical BMT and 6 months after LKT there are no signs of GvHD, the blood chimerism is 100%, the kidney allograft function is very good (GFR 73 ml/min/1.73 m<sup>2</sup>) and immunosuppression is withdrawn. Iron overload is being corrected by regular phlebotomies. The patient no longer requires antihypertensive medication and there is evidence of vascular remodeling. This is the first report of a successful haploidentical BMT followed by kidney transplantation from the same donor in a patient with SCD.

**Disclosure of conflict of interest:** None.

## P593

### Haploidentical transplantation with post-transplant cyclophosphamide for pediatric non-malignant disorders

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Allogeneic hematopoietic stem cell transplantation (HSCT) can cure non-malignant diseases, such as primary immune deficiency (PID), severe aplastic anemia (SAA) and osteopetrosis (OP). In the absence of a well-matched donor, transplantation from a haplo-identical donor may be considered. Post-transplant cyclophosphamide (PTCy) is a new strategy derived from the treatment of malignant diseases in adults that has been little studied in high-risk pediatric non-malignant diseases. Fifteen children (2.2 years, range: 0.19–10.88) underwent HSCT in the pediatric immunology and hematology unit of Necker Hospital, Paris, between December 2014 and September 2016. These children were suffering from OP ( $n=3$ ), SAA ( $n=2$ ), hemophagocytic lymphohistiocytosis (HLH) ( $n=3$ ), immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome ( $n=2$ ), combined immune deficiency ( $n=4$ ) and leukocyte adhesion molecule deficiency ( $n=1$ ). Three patients received a low-intensity conditioning regimen (CR) (based on fludarabine, cyclophosphamide, and total body irradiation) whereas the other 12 received myeloablative CR (based on busulfan AUC targeted and fludarabine). Fourteen patients received serotherapy before HSCT. Post-transplant cyclophosphamide (50 mg/kg/day) was given on D3 and D4 and graft versus host disease (GVHD) prophylaxis with cyclosporine and mycophenolate mofetil was initiated on D5. The transplanted stem cells were obtained from bone marrow in all cases. Engraftment with full donor chimerism was observed in 13 patients. The median CD34+ cell dose was  $14.8 \times 10^6$  cells/kg body weight (range:  $7.4\text{--}35.9 \times 10^6$ ). Neutrophils recovered after a median of 18 days (range: 14–32), and overall survival (OS) was 80% after a median follow-up of 1 year (range: 0.18–1.98). Three patients died due to graft failure ( $n=2$ ) or infectious complications related to GVHD ( $n=1$ ). Grade II acute GVHD occurred in 8 of the 13 patients displaying engraftment (62%), and chronic GVHD and/or autoimmune complications were observed in four patients (31%). Viral complications were frequent, occurring in 10 patients (77%) with CMV infection ( $n=8$ ) /disease ( $n=1$ ), adenovirus disease ( $n=1$ ) and BK virus cystitis ( $n=4$ ). Haploidentical transplant with PTCy is feasible in high-risk patients with non-malignant diseases. Chronic GVHD and autoimmunity were more frequent than for more conventional approaches in such patients. Infection rates were high.

**Disclosure of conflict of interest:** None.

## P594

### Hematopoietic stem cell transplant for sickle cell disease: The Indian experience

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Sickle cell disease (SCD) remains associated with high risks of morbidity and early death. Even best of supportive care fails to improve quality of life. Hematopoietic stem cell transplant (HSCT) can be considered for selected group of patients. In long run it is not just economical but also substantially improves quality of life (QOL). We report our experience with HSCT for SCD from India. Seventy three consecutive patients suffering from SCD who underwent HSCT between January 2006 and November 2016 were included in the study. Fifty two underwent matched sibling donor (MSD), 2 matched family donor (MFD), 3 matched unrelated (91/0 or 10/10), 2 cord blood transplant CBT (1 matched sibling cord blood and 1 matched unrelated) and 15 patient underwent haploidentical transplant. Different conditioning regimens were used and so was the graft versus host disease prophylaxis depending on institutional protocols as depicted in table 1.

**Table 1: Type of HSCT, conditioning and GVHD prophylaxis:**

Type of HSCT	No. of Patients	Conditioning	GVHD prophylaxis
Matched sibling donor	51	Bu+Cy+ATGAM Flu+Cy+TBI+TT+ATGAM Flu+Bu+TT+ATGAM Cy+TBI Flu+Bu+ATGAM Flu+Treo+TT+ATGAM Flu+Treo+TT	CSA+MTX PTCy+TAC CSA+TAC+MMF CSA+MTX+CY+Prednisolone TAC+MTX
Matched family donor	2	Flu+Treo+TT Flu+Treo+TT+ATGAM	TAC+MTX
10/10 and 9/10 Matched unrelated donor	3	Flu+Treo+TT+Thymoglobulin Flu+Bu+ATGAM Bu+Cy+ATGAM	CSA+MTX TAC+MTX
Cord blood transplant (matched sibling / matched unrelated)	2	Bu+Cy+ATGAM Flu+Treo+TT+ATGAM	CSA+MP TAC+MMF
Haploidentical family donor	15	Flu+Cy+TBI+TT+Thymoglobulin Flu+Bu+TT+Thymoglobulin Flu+Cy+TBI+Thymoglobulin Flu+Treo+TT+Thymoglobulin Flu+Treo+TBI	PTCy+TAC+MMF PTCy+TAC TAC+MMF

A total of 73 patients underwent SCT. The median age was 9 years (10 months-29 years). M/F ratio was 45/28. Majority of patients were either from African union or Oman. All patients suffered from one or other severe symptoms making them eligible for SCT. Graft source was bone marrow (BM) in 30 with median CD34 count of 5.3x106/kg (0.92-10.7), peripheral blood (PB) in 36 with median CD34 count of 8.5x106/kg (3.9-20.18), cord blood in 2 with median CD34 count of 1.27x105/kg (0.44-2.1) and combined BM & PB in 5 with median CD34 count of 6.37x106/kg (1.5-23.3). Of the 73 patients, 61 are alive and disease free with Lansky/Karnofsky scores of 100. There were 8 deaths (4 MSD/MFD/MUD; 3 haploidentical and 1 matched unrelated CBT). Four patients rejected the graft (2 haploidentical and 2 MSD/MFD/MUD). At the last follow up, the probabilities of survival, SCD-free survival, and transplant-related mortality were 89%, 83.5%, and 11%, respectively. Outcome of HSCT in SCD has improved significantly. With better conditioning regimens, improved supportive care, the outcome of alternative donor transplant and adult SCD has improved and matches sibling donor transplant. HSCT should be strongly considered as a curative modality for selected patients suffering from SCD.

**Disclosure of conflict of interest:** None.

#### P595

##### **Hematopoietic stem cell transplantation for children with thalassemia: A start up cooperative project in Iraqi Kurdistan**

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M Maria Speranza<sup>3</sup>, N Diana<sup>1</sup>, F Andrea<sup>3</sup>, P Marco<sup>3</sup>, S Hiwa<sup>1</sup>, M Dara<sup>1</sup>, A Kosar<sup>1</sup>, M Ignazio<sup>3</sup>, Q Sergio<sup>4</sup> and O Dosti<sup>1</sup>  
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Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment for thalassemia major which is now accepted as clinical standard, with approximately 1400 transplants performed after the year 2000 in 128 centers over 23 countries. However, not all the countries have enough resources and expertise to establish an HSCT program, and patients are often forced to seek transplantation abroad, with heavy social and economic consequences. In Kurdistan there are more than 2500 thalassemia cases recorded and nearly 150 of them have been referred abroad by Kurdistan Government coverage of all the costs. In 2015 the Italian Development Cooperation Agency identified the Hiwa Cancer Hospital (HCH) in Sulaymaniyah (Iraqi Kurdistan) as a possible site for the establishment of a new HSCT transplant center, due to a number of factors, namely availability of an good health system in Sulaymaniyah which ranked first in Iraq in last decade, availability of a unit including 6 HEPA filtered positive pressure rooms, a well established thalassemia center which serves nearly 1000 thalassemia patients, and finally a well equipped HLA laboratory. The aim of the cooperation activities was to establish an allogeneic transplant program for children with thalassemia having a matched sibling donor. Since April 2016 a team of international experts have been providing education and training of local staff. International and local

staff jointly defined more than 50 local standard operating procedures. Patients with low-risk characteristics (age  $\leq 7$  years, liver size  $\leq 2$  cm below costal margin) and a HLA matched sibling donor were considered eligible in this initial phase of activity. A downstaging protocol with hydroxyurea and deferoxamine or deferasirox was adopted. Conditioning regimen included iv busulfan and cyclophosphamide. GvHD and rejection prophylaxis included ATG from day -12 to -10 and CsA, MTX and methylprednisolone. GCS-F primed bone marrow was chosen as stem cell source. The first allogeneic HSCT of the whole Iraq was performed in a child with thalassemia at Hiwa Hospital in October 2016. Up to now, 3 patients (2 females, 1 male) underwent HSCT; median age at transplantation was 2 years; median infused TNC  $18.5 \times 10^8$ /kg, CD34+  $10.1 \times 10^6$ /kg. All of them engrafted. No major complication were observed. One of them developed grade II aGvHD (skin only) which resolved after increasing the dose of steroids. A huge number of patients with low-risk thalassemia are now in the waiting list and some of them have already started downstaging having planned HSCT in a short time. A matched sibling transplant program in children with thalassemia is feasible and safe in Kurdistan. Such a program can provide many advantages: far less psychosocial and financial burden for the families and significant saving for the government. The estimated costs of performing locally HSCT are much less than in the countries where patients were previously referred. The continuation of cooperation is of paramount relevance for further implementing the activity and extending the transplant accessibility to patients with other hemato-oncological disorders of childhood.

**Disclosure of conflict of interest:** None.

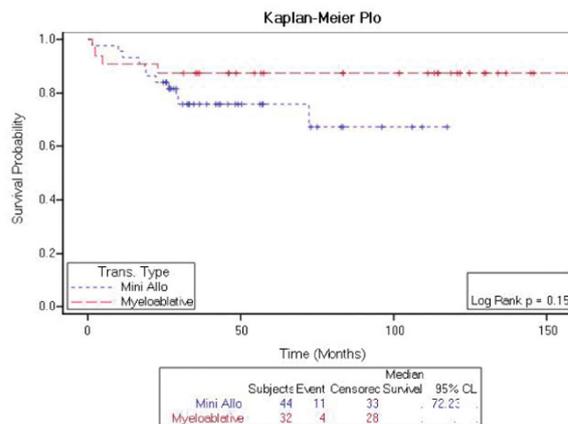
#### P596

##### Long term follow-up after reduced-intensity conditioning and stem cell transplantation for Thalassemia major

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Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for thalassemia major (TM). Reduced-intensity conditioning (RIC) before HSCT for high risk TM patients results in fewer complications, when compared with myeloablative regimens. One hundred and three TM patients received HSCTs from an HLA-identical related donor at King Hussein Cancer Center, between January 2002 and November 2016. Of those, 62 were high risk TM (60%) who received RIC HSCTs. In this report, we describe follow-up beyond 2 years (median, 42; 24.6–126 months) post RIC HSCTs. Forty-four class II–III patients (58%) were identified (25% with hepatitis C); with a median age of 14 (13.4–28) years. Females accounted for 59% ( $n=26$ ). Conditioning regimen consisted of oral busulfan 8 mg/Kg, fludarabine 175mg/m<sup>2</sup>, TLI 500cGy and ATG followed by PBSCT. GvHD prophylaxis consisted of MMF and CSA. Median infused stem cell dose was  $5.4 \times 10^6$ /Kg. All patients attained neutrophil and platelet engraftment (median, 15.3 and 21.3 days, respectively). Persistent mixed donor or full donor chimerism were observed in 95.5% ( $n=42$ ) and 4.5% ( $n=2$ ), respectively. Immune-suppressive therapy for GVHD treatment was required in 16 (36.4%) patients (aGVHD,  $n=8$ ; cGVHD,  $n=8$ ). Moreover, veno-occlusive disease occurred in 4 patients (9%) that resolved completely. Secondary graft failure was noted in 11 (25%) patients. The 5-year overall survival was 100%, while the 5-year probability of thalassemia-free survival was 72.2%. Other factors evaluated include: growth parameters, endocrine and other organ functions, in addition to functional status. This report confirms the safety and efficacy of RIC regimens in HSCTs for high risk TM patients. Those regimens are associated with excellent engraftment and sustained mixed donor chimerism; and lead to excellent thalassemia-free and overall survival rates.

[P596]



**Disclosure of conflict of interest:** None.

#### P597

##### Long-time follow-up and late effects estimation in patients with Hurler syndrome after allogenic hematopoietic stem cell transplantation: Results of Russian joint study

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In-time HSCT for pts. with Hurler syndrome (HS) can significantly improve the results. Long-term follow-up and late effects estimation required to prepare a special observation and rehabilitation programs. Aim. To analyze our experience with HSCT for HS in the field of special observation and rehabilitation programs. Forty HSCT during the 2004–2016 were performed for 38 pts. with HS. Median age at the diagnosis was 15 months (3–38 months), at HSCT—24 months (9–48 months). BM used in 62.5% ( $n=25$ ), PBSC—32.5% ( $n=13$ ), CB—5.0% ( $n=2$ ). MAC conditioning was used for 29 HSCT, RIC—for 11. RIC regimen: Flu+Mel+ATG, MAC: Bu/Treo+Flu+Thio/Mel (Bu was used in early 2000) and ATG +Rituximab (in case of MUD HSCT). All pts. with RIC received MUD HSCT, pts. with MAC MUD—18 pts., MRD—5 pts. Pts. received CsA/Tacro-based GvHD prophylaxis. MMF/MTX was additionally added in all cases. In 3 RIC HSCT immunomagnetic CD3/CD19+ depletion of PBSC (by CliniMACS) was used. A special observation protocol including somatic and neurocognitive estimation was developed. All pts. engrafted with full donor chimerism on D+30. Median of engraftment day—20 (11–29 days). Thirty three pts. survived. Reasons of death—MAC: infections—3 pts., RIC: TRALI—1 pt., aGvHD—1 pt. TRM improved, over the years, with improving of supportive care and donor selection as well as pre-transplant screening. No early severe toxicity revealed. Pulmonary infection episodes was registered in 30% of pts. in our study. GvHD: Grade II—developed 14 pts., Grade III–IV—2 pts. (after RIC), local cGVHD—3 pts. (RIC). No extensive cGVHD. 5 pts. rejected (MAC and RIC rejection rate was same). At median follow up of 60 months (8–160 months), the estimated 5 years pOS was 81%. Best response correlated with early HSCT (and better status before HSCT) and higher level of AIDU after. Late effects estimation showed that 47.4% ( $n=18$ ) of patients experienced late effects: cardio-vascular—16 pts., skeletal—15 pts., endocrine—3 pts. All pts. with cardio-vascular effects received MAC. Skeletal

effects affected patients of older age, pts. transplanted in younger age do not have such effects. Median period of late effects arising after HSCT was 15 month (3–27 months). Only 3 pts. experienced serious pulmonary late effects (infections), all episodes was before 2010. No pts. in our study have progressive retinal degeneration. 80% of pts. improved in the neurosensory component and all pts. improved in neurocognitive status and development after HSCT. Best response correlated with neurocognitive rehabilitation based on unique computer model used by our group in Russian national rehabilitation center “Russkoe pole.” In-time HSCT is an effective and safe way to stop neurodegenerative process for pts. with HS. Both MAC and RIC regimens can be used with the same effectiveness. MAC regimens associated with bigger number of cardiovascular late effects. Long-time follow-up showed that these patients require the special observational protocol including estimation of cardio-vascular, skeletal, endocrine and neurocognitive risks. Better neurocognitive response correlated with intensive rehabilitation using computer model. Russian Joint study showed effective cooperation for treatment pts. with HS in the national setting.

**Disclosure of conflict of interest:** None.

#### P598

##### **Non PTLD malignancy post-HSCT for primary immunodeficiency—A case series**

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Treatment for Primary Immunodeficiency (PID) has evolved with more patients undergoing and surviving haematopoietic stem cell transplantation (HSCT). Estimated overall malignancy risk for patients with PID is 4–25%. We report 12 cases who developed non-PTLD secondary malignancy post-HSCT for PID. Cases were evaluated regarding PID diagnosis, timing and type of transplant, conditioning regimens, type of malignancy and outcome from the 2 UK PID referral centers. 12/944 patients (1.27%) developed non-PTLD malignancy. Median interval from HSCT to malignancy diagnosis was 3.75 yrs (3 months–11.2 years).

T=Treosulfan, F=Fludarabine, Al=Alemtuzumab, Mel=Melphalan, Bu=Busulfan, Cy=Cyclophosphamide, MSD=matched sibling donor, UCB=Unrelated Cord Blood, MUD=Matched Unrelated Donor, BM=bone marrow, PBSC=Peripheral Blood Stem Cells JMML=Juvenile Myelomonocytic Leukemia, AML=Acute Myeloid Leukemia, RMS=Rhabdomyosarcoma, Ca=Carcinoma, MEC=Mucopidermoid Carcinoma  
Discussion: Whilst early haematological malignancies likely relate to the original molecular defect in remaining recipient cells, most of our patients developed late rare solid tumors. Little is known about pathogenesis of solid tumors after HSCT but, intensive cytotoxic conditioning therapy with defective DNA repair of persisting stem cells/stromal cells, viral infection, and immunosuppression may play a role. 3/6 patients with solid tumors had a Melphalan-based conditioning. Melphalan was linked to sarcoma and lung cancer in animal model. There are few data linking parotid MEC to infection by CMV and HHV6 which can remain dormant in the salivary glands. Both affected patients had HHV6 during the transplant period. P8 and P11 had a family history of solid tumor pointing to a possible genetic factor. Whilst secondary malignancy post-HSCT for patients with malignant disorders is well recognised, non-PTLD malignancy post-HSCT for PID has not previously been reported. A larger study is needed to evaluate incidence and risks.

#### **References**

1. Filipovich AH, et al. *Cancer Res* 1992; **52**: 5465s.

**Disclosure of conflict of interest:** None.

#### P599

##### **Non-myeloablative alemtuzumab/low dose total body irradiation conditioning for children undergoing HLA-matched sibling donor haematopoietic cell transplantation for sickle cell disease**

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Haematopoietic cell transplant (HCT) is the only curative approach for SCD. Due to concerns regarding the toxicities associated with myeloablative conditioning regimens in adults, a non-myeloablative protocol was developed by Hsieh et al. (National Institutes of Health, NIH protocol). The use of this novel regimen was able to achieve a curative degree of mixed donor chimerisms with minimal transplant-related complications. The Alberta Children's Hospital (ACH) has adopted this conditioning regimen in children due to the efficacy and low rates of toxicities published by the NIH group. With generally lower rates of GVHD in younger recipients, our group had no reason to believe rates of toxicities would be greater in a younger population with fewer comorbidities secondary to SCD than those described in the NIH cohort. To our knowledge, there is no published literature describing the utilization of the NIH protocol in a paediatric population. We describe our experience in children with SCD who underwent matched sibling donor (MSD) peripheral blood HCT using NIH protocol. This retrospective cohort describes outcomes of MSD HCT in children with SCD who underwent HCT with the NIH conditioning regimen between 2013–2017. A total of 13 potential subjects were identified. Eight subjects have consented to the analysis to date. MSDs with either normal haemoglobin or sickle cell trait were considered appropriate for donation. The transplant procedure: The conditioning regimen consisted of alemtuzumab 0.2 mg/kg/dose administered subcutaneously daily for five days (Days -7 to Day -3). Patients received a TBI dose of 300 cGy on day -2, with testicular shielding for male recipients. GVHD prophylaxis consisted of a sirolimus load of 3 mg/m<sup>2</sup>/dose (PO) on day -1, followed by 1 mg/m<sup>2</sup>/dose once a day starting on Day 0. Unmanipulated peripheral blood stem cells were infused on day 0. Sirolimus was used for GVHD prophylaxis post-HCT and continued until at least one year. Weaning of sirolimus was initiated no earlier than 1 year post-HCT and if donor T-cell chimerisms were greater than 50%. Institutional supportive care protocols for SCD HCT were followed. Patients were eligible for early discharge post-HCT even prior to neutrophil engraftment. Eight patients (5 F, 3 M) have been registered on this retrospective study. Follow-up ranges from 1 to 52 months post-HCT. There were no failed stem cell mobilizations. All patients had donor neutrophil engraftment at a median of 21 days. All patients are currently alive. There have been no cases of graft failure to date and no sickling crises post-HCT. One patient has dropping myeloid chimerisms but still > 20% donor. No cases of veno-occlusive disease, idiopathic pneumonia syndrome, cerebral hemorrhage, PRES, or post-transplant lymphoproliferative disease were observed. Three cases of cytomegalovirus (CMV) reactivation required pre-emptive therapy. Only one patient did not initiate sirolimus weaning at 1 year post-HCT due to donor T-cell chimerisms of 42%; this patient is 52 months post-HCT and is likely to start weaning sirolimus soon. There have been no cases of acute or chronic graft-versus-host disease. Non-myeloablative conditioning regimen is safe and effective as curative therapy for SCD. Long-term follow-up of these children to assess organ function post-HCT is underway.

**Disclosure of conflict of interest:** None.

Diagnosis	Age@HSCT (months)	Conditioning Regimen	Source of stem cells	Chimerism T/B/Myeloid	Malignancy	Interval post HSCT (years)	Status
T-B-NK+ SCID	4	T/F	MSD BM	100/0/0	Ph+ Pre B ALL	2.5	Died
JAK 3 SCID	1.5	T/F/Al	UCB	100/100/100	Ewing Tumor	3.58	Alive
MHC I	180	T/F/Al	MUD BM	100/100/100	Parotid MEC	6	Alive
CID	168	T/F/Al	MSD BM	100/100/100	Parotid MEC	3	Alive
CID	191	F/Mel/Al	MUD BM	94/100/100	Toe Melanoma	6.9	Alive
WAS	204	F/Mel/Al	MUD BM	100/100/100	Sq cell ca (gastrostomy site)	0.25	Alive
XL-CGD	180	F/Bu/Al	MUD PBSC	100/100/100	Basal cell ca (ear)	4	Alive
CD40 ligand	21	F/Mel/Al	MUD PBSC	34/0/9	Renal cell ca	4.8	Alive
T+B+NK low SCID	12	Bu/Cy/Al	MUD BM	96% Whole blood	AML	11	Alive
LAD1	17	F/Mel/Al	MUD BM	100/100/100	Renal Cell Ca	11.2	Alive
T-B+NK+ SCID	53	F/Mel/Al	MSD BM	100/100/100	Embryonal RMS	0.45	Alive
Griscelli/HLH	8	T/F	MSD BM	0% whole blood	JMML	0.78	Died

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**Successful allogeneic hematopoietic stem cells transplantation from HLA-identical sibling selected with preimplantation genetic diagnosis (PGD) with HLA matching in a patient with Shwachman-Diamond syndrome (SDS)**

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Allogeneic HSCT is a treatment of choice for the bone marrow failure in patients with SDS. HSCT from unrelated or mismatched family donors is associated with higher morbidity and mortality compared with matched sibling. Combined PGD and HLA antigen testing is a possible option to preselect a compatible donor for an affected sibling requiring HSCT. We describe a case report demonstrating first successful HSCT for 6 years girl with SDS by using preimplantation genetic

diagnosis and HLA matching. Diagnosis of SDS was suspected at 5 m.o., based on clinical features, family history, laboratory studies. At 6 m.o., bone marrow (BM) aspiration revealed hypocellular marrow with signs of dysplasia and expansion of blasts (15.6% blasts). The Sanger sequencing of SBDS gene showed c.183-184TA>CT and c.258+2T>C mutations. The patient had recurrent infections, including bilateral pneumonia caused by Phaeoohyphomyces, bloodstream infection, CMV-disease. Due to the lack of matched related or unrelated donors, HSCT with RIC (Flu, Mel, ATG) from haploidentical father was performed at 16 months of age. After the 1st allo-HSCT, engraftment was achieved on D+13, initial STR study showed full donor chimerism. Post-transplant period was complicated with severe CMV-infection and signs of secondary HLH. At D+135, graft rejection was registered. The girl became dependent on regular RBC and platelet transfusions, BM examination revealed hypocellularity with moderate signs of myelodysplasia without elevated blast count. Due to lack of available HLA-compatible donors, an option of *in vitro* fertilization (IVF) with preimplantation selection of a normal HLA-matched embryo was considered. After controlled ovarian hyperstimulation 2 embryos were HLA-compatible and healthy (first, wild-type; second, heterozygous for SBDS gene mutation c.183-184TA>CT). Hence, the only unaffected HLA-identical embryo was transferred resulting into full-term pregnancy. At the age of 5.5 years after 1st HSCT, the 2nd

transplant was performed with a combination of CB and BM as a source of hematopoietic stem cells. The donors' age was 2 years. A reduced toxicity conditioning regimen (RTC) based on Flu150 mg/m<sup>2</sup>, Treo42 g/m<sup>2</sup>, Thiotepa 10 mg/kg with serotherapy (Thymoglobuline 7.5 mg/kg) was used. Because of neurotoxicity, arterial hypertension, since D+1 CsA was changed to Sirolimus +MMF for GVHD prophylaxis. The total number of infused NC was 2.5 × 10<sup>8</sup>/kg; CD34+, 3.85 × 10<sup>6</sup>/kg; CD3+, 2.4 × 10<sup>7</sup>/kg. Engraftment was achieved on D+28. Any signs of GVHD, severe infectious or toxic complications were not observed. Eight months later, the patient is alive, has full donor chimerism in BM and is not transfusion-dependent. In the absence of HLA-identical donor, IVF with preliminary PGD and HLA-typing could be a chance for matched donor to cure patients with non-malignant genetic diseases. In case of low cord blood cellularity, a combination of CB and BM from the same sibling could be used. Our experience showed a successful engraftment of SDS patient and stable donor chimerism after second HSCT of CB and BM from PGD-selected sibling with RTC.

**Disclosure of conflict of interest:** None.

**P601**

**The safety and efficacy of familial haploidentical (FHI) stem cell transplantation utilizing CD34 enrichment and CD3 addback in patients with high risk sickle cell disease (SCD) (IND 14359)**

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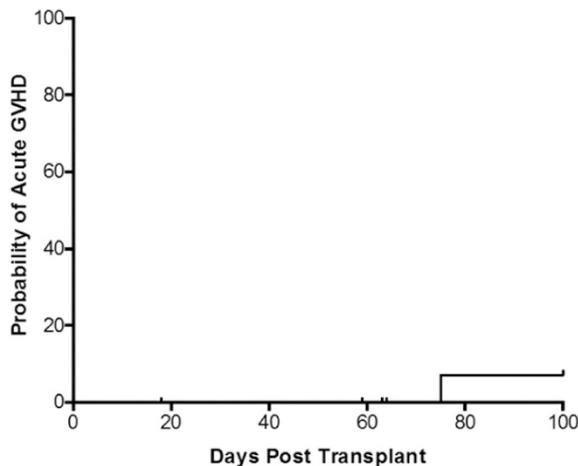
Sickle cell disease (SCD) is characterized by chronic vaso-occlusion, multi-organ failure and premature death.

Myeloablative conditioning (MAC) followed by HLA-matched sibling donor allogeneic stem cell transplantation (AlloSCT) is the only known curative therapy in patients with SCD, but less than 15% of patients with high-risk SCD have a HLA-matched sibling donor (Talano/Cairo et al, EJM 2014). Reduced toxicity conditioning (RTC) and AlloSCT results in 100% EFS and long-term donor chimerism in patients with SCD (Bhatia/Cairo et al, BMT 2014). However, RTC and UCBT resulted in an unacceptable rate of primary graft failure in patients with SCD (Radhakrishnan/Cairo et al, BBMT 2013). More recently, RTC and matched unrelated donor (MUD) T-replete AlloSCT has resulted in a 60% 2yr EFS but a high probability of CGVHD (62%) (Shenoy et al, Blood 2016). We previously developed a method of CD34 enrichment and CD3 addback in children of MUD recipients with sustained long-term donor chimerism and low probability of Grade II-IV AGVHD (Geyer/Cairo et al, BJH 2011). In this study we investigated the safety and efficacy of FHI and CD2 addback in patients with high-risk SCD. Eligibility: Patients 2-21 yrs, high-risk: CVA, ≥ 2 ACS, ≥ 3 VOC, or 2 abnormal TCDs. MAC: Hydroxyurea 60 mg/kg/day and Azathioprine 3 mg/kg/day, Day -59 - Day -11, Fludarabine 150 mg/m<sup>2</sup>, Busulfan 12.8 mg/kg, Thiotepa 10 mg/kg, Cyclophosphamide 200 mg/kg, R-ATG 8 mg/kg, TLI 500 cGy, followed by FHI, CD34 enrichment (Miltenyi) 10 × 10<sup>6</sup> CD34/kg and 2 × 10<sup>5</sup> CD3/kg (MNC) addback. Tacrolimus AGVHD prophylaxis. Eighteen patients enrolled and 16 patients have had AlloSCT to date. Median time to myeloid engraftment (10 days), ≥94% whole blood and ≥89% RBC enriched donor chimerism. Probability of Grade II-IV AGVHD is 7.1% (CI95: 0-69%) (Figure 1A). Probability of 1yr EFS is 87.4% (CI95: 58-97%) (Figure 1B). Immune cell reconstitution has been robust and similar to RTC and MSD AlloSCT in SCD (Table 1). There have been 3 deaths, VOD, steroid refractory AGVHD and CGVHD. MAC followed by FHI utilizing CD34 enrichment and T-cell addback in patients with high-risk SCD is safe, tolerable and results in long-term donor chimerism and absence of SCD symptoms or complications. A larger cohort and follow-up will be required to confirm these preliminary findings.

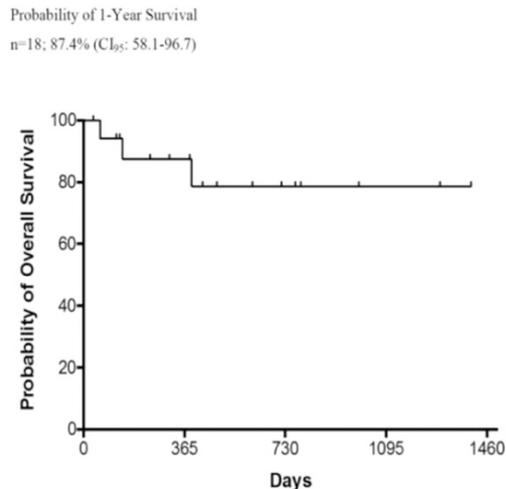
**Disclosure of conflict of interest:** None. Supported by R01FD004090-01A1.

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**Figure 1A. Probability of AGVHD: 15 evaluable patients; 7.1% (CI95: 0-69.0)**



**Figure 1b. Probability of 1yr Survival**



# Lymphoma

## P602

### A clinical prognostic index for assessing patients aged > 60 being considered for high-dose therapy and autologous stem-cell transplant in relapsed or refractory high-grade non-Hodgkin lymphoma

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Patients with relapsed high-grade NHL or disease refractory to first-line therapy can still be cured with high-dose therapy and autologous stem cell transplant if they respond to salvage chemotherapy. This aggressive algorithm is accepted in younger patients but is less well established in the elderly. Age > 60 has a negative predictive score in the International Prognostic Index (IPI) and there are concerns that the outcomes of HDT in these patients are significantly worse. Deciding which older patients will benefit from HDT is challenging and there are no established predictive tools to guide physicians. We present a clinical prognostic index derived from information readily available at the time a patient is being assessed for ASCT. The BSBMT audited the outcomes for UK patients aged >60 transplanted between 2004–2009 (n=371) and benchmarked against the European Bone Marrow Transplant (EBMT) database for the same period (n=2695). The primary outcome was progression-free survival (PFS) but data was also analysed for overall survival (OS), relapse rate (RR) and non-relapse mortality (NRM). We included all patients with a diagnosis of high grade NHL and the following demographic features were also analysed: Age at diagnosis; age at transplant; M/F; year of transplant; CR/Not CR at transplant; no. of prior therapies; no. of cells infused; clinical

staging; Karnofsky status at transplant; histology; IPI at diagnosis; mobilising regime and conditioning regime. Candidate prognostic indices were factors achieving significance in univariate and multivariate analyses of the main outcomes by regression analysis. The best prognostic index was selected based on the BSBMT dataset and then applied to the rest of the EBMT dataset (the validation dataset). There were no significant differences in patient characteristics between the UK and non-UK groups nor in outcomes of PFS, OS, RR or NRM. (Figure 1). In both univariate and multivariate analysis the following features were associated with a significantly worse outcome for PFS, OS, RR and NRM : Age > 66, Karnofsky Score.

**Disclosure of conflict of interest:** None.

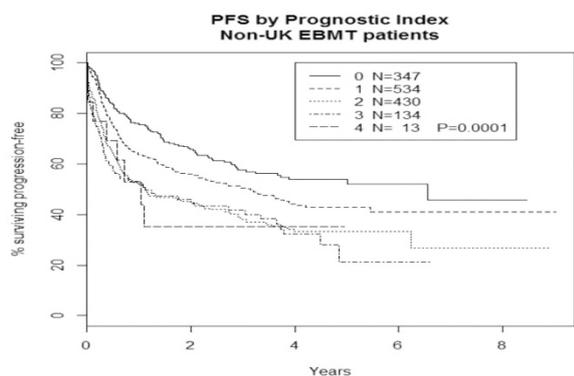
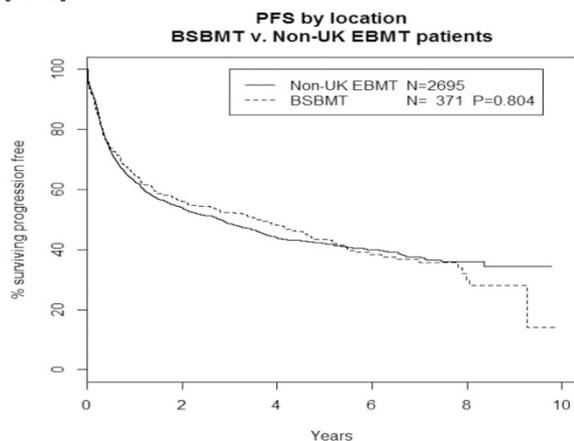
## P603

### Allogeneic stem cell transplantation (allo-SCT) after treosulfan-based conditioning regimen for B-cell non-Hodgkin lymphoma (B-NHL)

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Allo-SCT is nowadays considered an effective therapy in the management of relapsed and refractory B-NHL. Between June 2005 and September 2015, forty-one heavily pretreated patients (pts) affected by relapsed and refractory B-NHL (17 DLBCL, 10 FCL, 8 MCL, 4 BL, 1 PMBCL, and 1 CLL/SLL) underwent an allo-SCT at our center after a Treosulfan-based conditioning regimen. Eleven pts received a MRD, 19 pts a MUD, and 11 pts a HAPLO unmanipulated PBSC allo-SCT. At allo-SCT 17 pts were in CR, 10 pts were in PR, and 14 pts had SD/PD. HCT-CI was evaluable for 34 pts, 18 had a score  $\geq 3$ . The backbone conditioning regimen consisted of Treosulfan 14g/m<sup>2</sup> from day -6 to -4, and Fludarabine 30 mg/m<sup>2</sup> from day -6 to -2; twenty-five pts were treated with this Reduced Toxicity Conditioning (RTC) regimen. Intensification with other alkylating agent (Melphalan, Thiotepa, or Cyclophosphamide) or radiotherapy (4Gy total dose) was applied on the remaining 16 pts (Myeloablative Conditioning, MAC). GvHD prophylaxis was based on Cyclosporine A and Methotrexate (17 pts) or Rapamycin and Mycophenolate Mofetil (24 pts), plus anti-Thymocyte Globulin or post-transplant Cyclophosphamide accordingly to donor type. Median numbers of infused CD34 +/Kg and CD3+/Kg were  $6.66 \times 10^6$  (range: 2.72–12.24) and  $2.34 \times 10^8$  (range: 0.03–6.89), respectively. Median follow-up was 61 months (range: 18–139). Thirty-nine pts were evaluable for engraftment; median time to neutrophil  $\geq 0.5 \times 10^9/L$  was 16 days (range: 10–30), and 16 days (range: 10–59) to platelet  $\geq 20 \times 10^9/L$ . Treosulfan conditioning provided a CR in 3 and 6 pts respectively in PR and SD/PD at transplant. No graft failure was observed. One and 5 years Overall Survival (OS) was 58.5% and 52.6%, respectively. Progression Free Survival (PFS) and GvHD-free/Relapse-Free Survival (GRFS) were respectively 51.2% and 41.5% at 1 year, 44.3% and 23% at 5 years. One and 5 years Relapse/progression Incidence (RI) was 29.3% and 36.1%, respectively. Transplant Related Mortality (TRM) was 14.6% at 100 days, 19.5% at 1 year and for the entire follow-up. The 100-day Cumulative Incidence (CI) of aGvHD grade  $\geq 2$  was 4.9%; CI of moderate to severe cGvHD was 24.8% at 2 years. The outcome of pts in CR at 5 years was significantly better compared to that of pts with active disease in terms of both OS (82.4% vs 33.3%,  $P < 0.005$ ), PFS (68.6% vs 25%,  $P < 0.005$ ), GRFS (36.3% vs 12.5%,  $P < 0.005$ ), and RI (19.6% vs 50%,  $P < 0.05$ ). No statistical differences in OS, PFS, and RI were found when pts were stratified according to donor type and

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the use of RTC or MAC regimen. At last follow-up, 22 patients are alive and disease free; 3 of them obtained a durable CR using chemotherapy and/or DLI for disease progression after allo-SCT. Treosulfan-based conditioning regimen is effective and well-tolerated in patients with advanced B-NHL undergoing allo-SCT.

**Disclosure of conflict of interest:** None.

#### P604

##### **Autologous stem cell transplantation for systemic anaplastic large cell lymphoma. A retrospective analysis of the lymphoma working party (LWP) of the EBMT**

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Systemic anaplastic large cell lymphoma (sALCL) is a very infrequent well-defined histological entity that comprises around 11% of all T-cell non-Hodgkin lymphoma. In the absence of prospective clinical trials, autologous stem cell transplantation (autoSCT) is considered the standard of care as consolidation therapy after first line therapy for those patients not expressing the Alk protein (Alk neg sALCL) and for patients with relapsed disease. The objective of this retrospective analysis was to analyse the long-term outcome of patients diagnosed with sALCL and being treated with autoSCT during the course of the disease, making special emphasis on the potential impact of the administration of brentuximab vedotin (BV). Eligible for this study were patients 18 years or above with sALCL who underwent autoSCT between 2010 to 2014 and were reported to the EBMT. Baseline patient, disease, and transplant data were collected from EBMT MED-A standard forms. Centers with potentially eligible patients were contacted to provide additional treatment and follow-up information including a written histopathology report for central review. Seventy-nine patients (48 males) with a median age at diagnosis of 43 years (range: 14–70) and at transplantation of 45 years (19–71) were included in the final analysis. Thirty-nine patients were Alk negative, 38 Alk positive and in 2 patients expression of Alk protein was unknown. At diagnosis, 60 patients (76%) presented with advanced stage and 48 (61%), with B symptoms. Sixty-three patients (80%) received 1–2 lines of therapy before autoSCT. Ten patients were treated with BV at some point before autoSCT; two patients as second line therapy, three as third line, one as fourth line and four as fifth line therapy. The median number of BV doses was 5 (range: 3–8). The median time between diagnosis and transplantation was 12 months (range: 4–233). Most patients had chemosensitive disease at autoSCT [65 patients (82%)] and in all but 2 patients peripheral blood was used as the source of stem cells. Conditioning regimen consisted on BEAM / BEAM-like protocols in 72 patients (91%). All patients engrafted. With a median follow up for surviving patients of 34 months (range: 2–71), 57 patients are alive (72%), 20 patients died (25%) and 2 patients (3%) are lost for follow up. Disease relapse after transplantation was the most frequent cause of death after the procedure. Cumulative incidence of non-relapse mortality for the whole series was 3% (95% CI, 0.5–9) at 100 days, 1 year and 3 years. Cumulative incidence of relapse was 27% (95% CI 17–27) and

32% (95%CI 21–44) at 1 and 3 years, respectively. 1 and 3-years progression free survival (PFS) was 70% (95% CI 60–82) and 65% (95% CI 54–78), respectively and 1 and 3-years overall survival (OS) was 82% (95% CI 73–91) and 71% (95% CI 61–83), respectively. There were no significant differences in any of the outcomes between BV treated and non-treated patients. AutoSCT results in a promising PFS and OS in patients with sALCL. The potential impact of the administration of BV as salvage strategy before the procedure needs to be further elucidated.

**Disclosure of conflict of interest:** None.

#### P605

##### **Autologous stem cell transplantation in refractory coeliac disease type II—The Irish experience**

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Coeliac disease (CD) is a T-cell immune-mediated enteropathy to dietary gluten, characterized by small bowel villous atrophy resulting in malabsorption. The enteropathy is reversible with a gluten-free diet (GFD), however symptoms and signs which persist > 1 year are defined as refractory coeliac disease (RCD). RCD is divided into type I and II, depending on absence/presence respectively of clonal intra-epithelial T-lymphocytes (IELs) with an aberrant phenotype (cytCD3 pos, membranous CD3, CD4 and CD8 neg). RCDII patients have a 5 year survival of 1.0, Plts > 20) was successful at a median of 11.5 (range: 10–15) days and no transplant-related mortality occurred. All patients achieved a clinical complete remission, with normalization of nutritional indices at 100 days, but persistently abnormal IELs and clonal T-cells on duodenal biopsy. With a median follow-up of 42.5 (range: 22–56) months, 5 patients remain in clinical remission, 1 patient relapsed with RCD and no patient progressed to EATL. Chemotherapy and ASCT is a safe and effective strategy for the treatment of RCD offering the possibility of sustained clinical responses. Clonal TCR in duodenal biopsy/blood and IEL flow cytometry form part of the patient evaluation prior to the chemotherapy/ASCT program.

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**Disclosure of conflict of interest:** None.

#### P606

##### **Autologous stem cell transplantation in refractory or relapsed Hodgkin lymphoma**

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Most patients with Hodgkin Lymphoma (HL) are cured with conventional chemotherapy. However, approximately 20% of patients relapse after primary treatment. For those, high-dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard of care. Fifty seven

[P606]

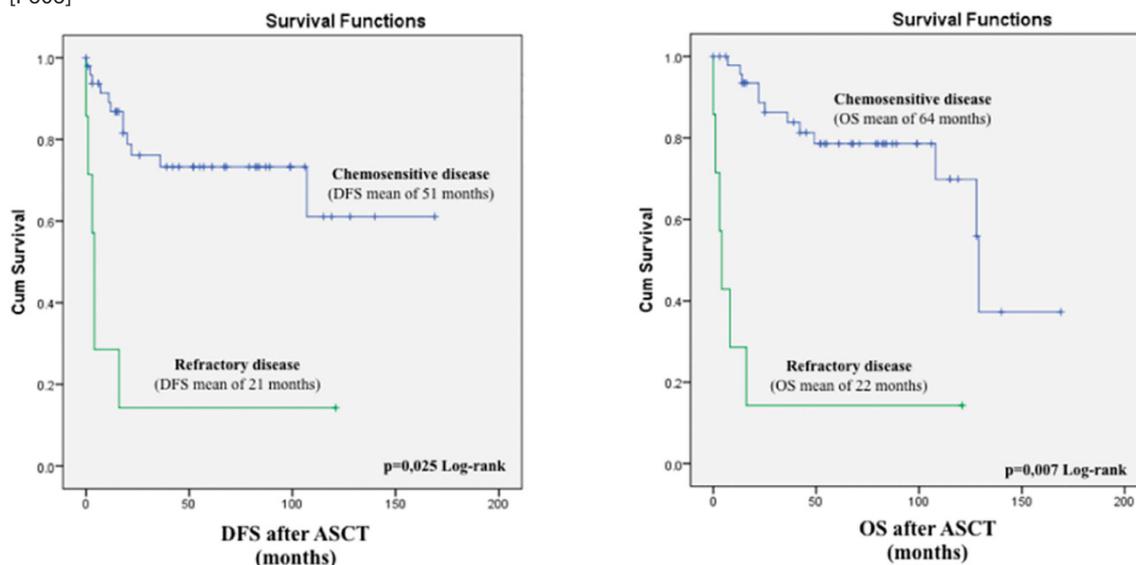


Figure 1: DFS and OS according to treatment response prior to ASCT

adult patients with relapsed or refractory HL submitted to ASCT between 2000 and 2015 were reviewed. Variables examined were sex, age, Ann Arbor stage (I-II vs III-IV), B symptoms, bulky disease, extranodal involvement, nodal areas involved ( $\geq 3$  vs  $\leq 12$  months) and response to the treatment prior to ASCT. Log-rank test was used to compare differences in survival for each factor. Patients median age was 31 (17–64) years at diagnosis. Ann Arbor stage III–IV in 35 (61%) patients, B symptoms in 25 (44%), extranodal involvement in 20 (35%) and bulky disease in 13 (23%). All patients were treated according to the ABVD protocol in first line. Indications for ASCT were relapsed disease ( $n = 30$ , 52.6%) and lack of complete response (CR) or progressive disease with 1st line treatment ( $n = 26$ , 45.6%). There were a median of 2 (2–5) treatment-lines before ASCT (protocols ESHAP, ICE, BEACOPP, GVD and others). The disease was chemosensitive in 86% cases: CR in 24 and partial response (PR) in 25 patients prior to ASCT. Refractory disease (RD) in 14% ( $n = 9$ ). In 84.2% patients, the hematopoietic cells mobilization was performed under stimulation with granulocyte-colony stimulating factor in hematologic recovery after the cycle of 2nd line chemotherapy, and most of which required 1 (1–4) apheresis. Conditioning regimens were BEAM (93%) and GMB (7%). The median time to hematologic recovery was 11 days (8–14) for neutrophils  $> 500/\mu\text{L}$  and 13 days (9–25) for platelets  $> 20,000/\mu\text{L}$ . Three months after ASCT, thirty-nine (68.4%) patients had CR, one (1.8%) patient maintained PR and 6 (10.5%) patients had disease progression. Status unknown in 7 patients and four (7%) patients died. Relapse rate 32% ( $n = 15/47$ ). With a median follow-up time after ASCT of 52 (0–169) months, median disease-free survival (DFS) was 26 (0–169) months and overall survival (OS) was 52 (0–169) months. There were 19 deaths (33.3%), four (7%) related to early infectious complications of ASCT, two (2.6%) due to late infectious complications, eleven (21.1%) due to disease progression and 1 (1.8%) in context of secondary Acute Myeloid Leukemia. Response to the treatment prior to ASCT was the only factor with survival influence. The DFS and OS differed significantly in chemosensitive disease compared with RD (DFS mean: 51 vs 21 months,  $P = 0.025$ , OS mean: 64 vs 22 months,  $P = 0.007$ ). The response to salvage treatment prior to ASCT is the main prognostic factor for survival after ASCT. Prognosis remains poor in patients with RD or early and disseminated relapses. For these patients, the therapeutic approach should include

intensive treatment with tandem HDC and stem cell transplantation, allogeneic transplant or early consolidation with brentuximab-vedotin after ASCT.

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**Disclosure of conflict of interest:** None.

#### P607

#### Bendamustine: Promising activity in post-brentuximab failure Hodgkin's lymphoma patients who had also failed a prior autologous HCT and possible successful bridging to allogeneic HCT

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Hodgkin's lymphoma (HL), although considered a curable neoplasm in adults, could be associated with a very poor prognosis when refractory to primary induction therapy or when it relapses within 12 months from an autologous stem cells transplant (auto-SCT). The optimal treatment of patients with heavily pretreated/refractory HL is controversial. Brentuximab vedotin (Bv) is an active single agent in this context; unfortunately, there are no well established therapies when patients fail to respond or progress after Bv. Encouraging results were recently described with checkpoint inhibitors. Similarly, data pertaining to efficacy of bendamustine (Benda) shows encouraging activity in various refractory lymphomas. We included in this study adult patients with HL who relapsed post auto-SCT and were refractory to or progressed after salvage Bv and were treated with Benda as salvage therapy with an intention to proceed with an allo-SCT. This study was

conducted in two major centers in Lebanon, the American university of Beirut Medical Center (AUBMC) and Makassed university hospital. We identified 12 eligible cases. The primary study endpoint was objective response rate (ORR). The secondary endpoint evaluated successful rate of bridging into an allo-SCT. The median follow-up times from Auto-SCT and from Benda salvage were 35 (14–59) and 10 (4–35) months, respectively. The median age of patients was 27 years (17–54). All patients had Bv as salvage therapy post Auto-SCT, and all of them progressed after a median of 4 (3–6) cycles. Clinical characteristics are outlined in Table 1. Patients received a median of 6 cycles (2–8) of Benda. The treatment was well tolerated, with rather infrequent adverse events and transient and manageable toxicities. The ORR was 75%, in 9 of 12 patients, with 43% obtaining a complete response. Eventually, 6 of 9 proceeded to allo-SCT using a matched related donor, and the remaining 3 patients are planned for allo-SCT. Only one patient died from disease progression after 24 months post allo-SCT. Two of 3 patients who progressed following benda received salvage therapy with nivolumab and are being planned for haplo-identical transplant while the third one is being planned for therapy with nivolumab. From the initiation of benda, the median duration of response for the 9 patients was 10 months (4–29); all these patients had maintained a continuous response at the last follow-up examination. Conclusion: Notwithstanding the limitations associated with our analysis, namely a small sample size and its retrospective nature, these results suggest a role for bendamustine in post Bv failures. These findings also provide the basis to evaluate the concept of Benda as a bridge to allo-SCT in a large prospective study.

[P607]

**Table 1. Patients' characteristics**

<b>Age</b>	27 (17–54)
<b>Gender</b>	
Male	8 (67)
Female	4 (33)
<b>HL subtypes</b>	
Nodular sclerosis	3 (25)
Classical	9 (75)
<b>Prior auto-SCT</b>	12 (100)
<b>Lines of chemotherapy pre benda</b>	5 (4–7)
<b>Frontline ABVD</b>	12 (100)
Primary refractory	3 (25)
Responded	8 (67)
Unknown	1 (8)
<b>Disease status at benda</b>	
Progressive disease	11 (92)
Partial response	1 (8)
<b>Brentuximab cycles</b>	4 (3–6)
<b>Interval auto relapse months</b>	13 (3–27)

**Disclosure of conflict of interest:** None

**Disclosure of conflict of interest:** None.

## P608

### **Brentuximab vedotin for relapsed or refractory Hodgkin lymphoma, single center experience king faisal specialist hospital and research center, Riyadh, Kingdom of Saudi Arabia**

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Hodgkin lymphoma (HL) patients with relapsed or progressive disease after high dose chemotherapy (HDC) and auto-SCT have limited curative options. FDA granted approval of brentuximab vedotin (BV) for the treatment of HL and anaplastic large cell lymphoma (ALCL) patients who fail auto-SCT or have had at least 2 prior multiagent chemotherapy regimens and are not candidates for auto-SCT. We are reporting single center experience of BV usage in this "approved" setting. Medical records were reviewed to collect required data. Kaplan–Meier (KM) method was used to calculate overall survival (OS) and progression free survival (PFS) from date of first dose of BV. From 2013–2015, 25 patients received BV. 24/25 had HL (22 classic HL-nodular sclerosis, 2 HL-mixed cellularity) and 1 ALCL. 19/25 (76%) pts were primary refractory or had early relapse after initial treatment. 15/25 (60%) pts received BV were refractory to the last treatment. All the baseline characteristics of patients are mentioned in Table 1. Median BV cycles administered were 6 (2–16). Overall response rate (ORR) was 40% (10 patients): CR in 6 (24%), PR in 4 (16%) (5/10 were primary refractory or early relapsed). Median PFS for whole group was 5 months (95% CI, 3.6–6.3). KM estimated 1-year OS was 74% and 2 year was 68%, median OS has not been reached yet. For 10 patients who responded, PFS at 12 months was 68% (95% CI, 38%–98%), median PFS not reached. For 15/25 patients with progressive disease (PD) or non responders after BV, median PFS was only 3 months (95% CI, 1.1–4.8). There was no difference in OS between patients with responders and non responders. Median OS has not yet been reached in either group as mentioned in survival curves. At the median follow up of 19 months (range: 4–55 months) 18 patients are alive, 10 patients are alive without disease, 4 patients received consolidation bone marrow transplant (2 auto-SCT and 2 allo-SCT). 2 patients completed 16 courses and achieved CR. Rest of 4 patients who are alive without disease; they had PD on BV but achieved CR with other treatments. 8 patients are alive with disease; 1 patient is on BV and 7 are on another treatment. 7 patients have died, 2 because of pneumonia while being on BV and 5 due to PD. 3/15 patients who received BV, achieved CR after failing all previous treatments and are in CR. Peripheral sensory neuropathy developed in 3 patients; one required dose reduction. 1 patient stopped treatment due to pulmonary toxicity. We are reporting largest single center data from Middle East which confirms that BV as a single agent is effective and safe. Overall response rate is lower as compare to pivotal trial but CR rate is comparable to other reported case series. This analysis also concludes that BV can be used as bridge to transplant in patients who don't respond salvage chemotherapy.

**Disclosure of conflict of interest:** None.

## P609

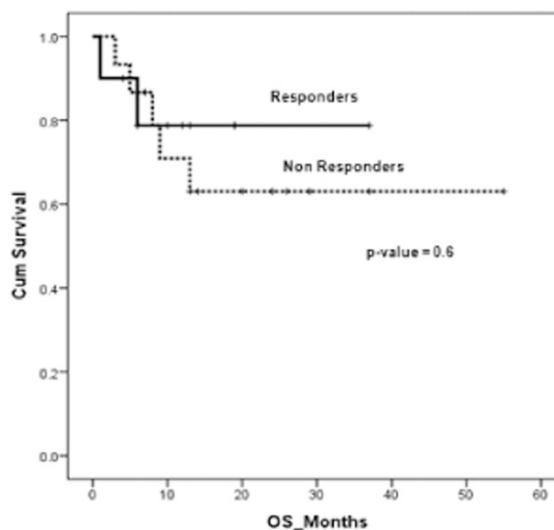
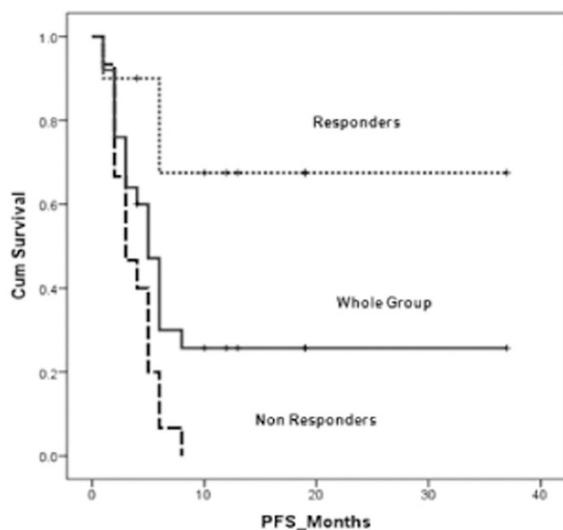
### **Effect of HIV infection on transplant outcomes after autologous peripheral blood stem cell transplantation: A retrospective study of Japanese registry data**

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Table 1

<b>Patient characteristics</b>	No=25 patients (%)
<b>Patient characteristics at diagnosis</b>	
Median age	27
Male: Female	1.5:1
Stage III/IV at first diagnosis	20 (80%)
Median number of previous cancer regimens, n (range)	3.2(range, 1–5)
Previous SCT(auto and allo SCT),prior to BV	18 (72%)
<b>Patient characteristics at BV administration</b>	
Median age	31
Stage III/IV	20 (80%)
Median number of BV courses	6 (range 2–16)
ECOG performance status	
1	16
2	8
3	1



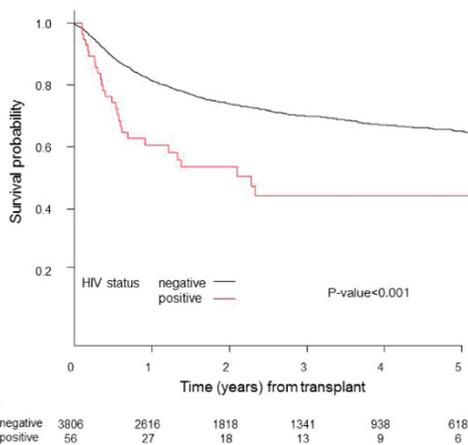
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The number of new HIV/AIDS cases has been declining in developed countries, whereas it is still increasing in Japan,

with the cumulative number reaching 26,607 as of June 28, 2016. HIV infection is associated with an increased risk of hematological malignancies such as non-Hodgkin lymphoma (NHL). Autologous hematopoietic cell transplantation (auto-HCT) is a treatment option for HIV-infected patients with NHL and multiple myeloma (MM). However, the prognosis after auto-HCT in HIV-infected Japanese patients remains unclear. The aim of this study is to evaluate the effect of HIV infection on transplant outcomes after auto-HCT in Japan. Using the national database of the Japan Society for Hematopoietic Cell Transplantation, we retrospectively evaluated patients with NHL ( $n=3862$ ) and MM ( $n=2670$ ) who underwent their first auto-HCT between 2001 and 2014. Presence of HIV antibody

was used to diagnose HIV infections. Cox proportional hazards models were used to evaluate risk factors of overall mortality. Fifty-six patients with NHL (1.4%) and 23 patients with MM (0.8%) were positive for HIV antibody. In patients with NHL, overall survival was significantly lower in the HIV-infected patients than in the HIV-negative patients [5-year overall survival: HIV-infected patients, 44% (95% confidence interval, 29%–58%) vs. HIV-negative patients, 65% (95% confidence interval, 63%–67%),  $P < 0.001$ ]. In a multivariate analysis, HIV infection was significantly associated with an increased risk of mortality (hazard ratio 2.39,  $P < 0.001$ ), and this effect was consistent regardless of transplant year. On the other hand, overall survival in patients with MM was similar between the 2 groups [61% (95% confidence interval, 31%–82%) vs. 63% (95% confidence interval, 63%–67%),  $P = 0.988$ ]. Previous studies in Europe and the United States showed comparable survival rates between HIV-infected and HIV-negative patients with NHL. However, our study showed that HIV infection was associated with a higher risk of mortality in patients with NHL in Japan. Suppression of T cell-mediated immunity or HIV related diseases might affect transplant outcomes in Japanese patients.

[P609]



**Disclosure of conflict of interest:** None.

**P610**

**Efficacy and toxicity of BEAC versus BEAM high-dose chemotherapy in patients with mantle cell lymphoma undergoing autologous stem cell transplantation**

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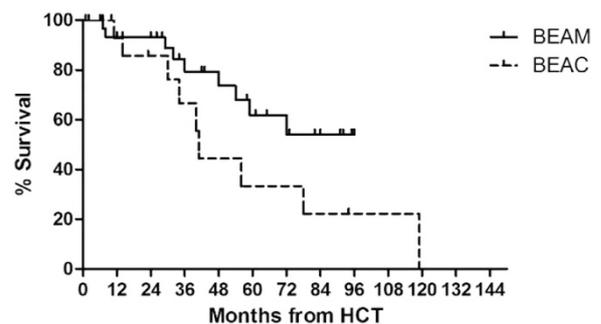
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While BEAM and BEAC regimens (BCNU, etoposide, cytosar in both regimens and melphalan or cytoxan, respectively) are commonly used as conditioning high-dose therapy (HDT) in patients with non-Hodgkin lymphoma (NHL), there have been few reports comparing these regimens. A retrospective analysis found the superiority of BEAM over BEAC in terms of overall survival (OS) and event-free survival (EFS). Toxicities were similar, except that BEAM was associated with more frequent lower gastrointestinal (GI) mucositis. Other studies reported that these regimens had similar efficacy and outcome. Recently, a concern regarding cardiotoxicity of BEAC has risen. The current study aimed to compare efficacy and toxicity of BEAC and BEAM as consolidation HDT in young patients with mantle cell lymphoma (MCL) undergoing autologous stem cell transplantation (ASCT). This is a retrospective analysis of outcomes in MCL patients who received HDT with BEAM or BEAC followed by ASCT at 3 bone marrow transplant centers in Israel. OS, disease-free survival (DFS) and progression-free survival (PFS) and regimen toxicity were compared. Seventy seven MCL patients who were diagnosed between

1995-1/2016 and received consolidation with BEAC or BEAM were included in the analysis. Forty nine patients were treated with BEAM and 28 patients with BEAC. No significant differences between the groups were revealed in terms of age, sex, the Mantle Cell Lymphoma International Prognostic Index (MIPI) risk score, induction protocol and% of patients transplanted in first complete response (CR1) (mean age 57 yrs in BEAM vs 59 yrs in BEAC group; 69% of patients in BEAM group had MIPI risk score 2–3 vs 62% in BEAC group; 68% of patients in BEAM group were transplanted in CR1 vs 71% in BEAC group). The amount of infused CD34 cells was significantly higher in the BEAM group (median CD34 cells/kg: 8.2 in BEAM vs 4.6 in BEAC groups;  $P = .001$ ); the number of days to platelet engraftment was significantly greater in the BEAC group (median 12 days in BEAM vs 14 days in BEAC group;  $P = .02$ ). There were no differences in the number of blood transfusions or hospitalization days between the groups. The rate of grade 3–4 upper mucositis was significantly higher in the BEAM group (41% in BEAM vs 18% in BEAC group;  $P = .046$ ); no other differences in toxicity (grade 3–4 lower mucositis, pulmonary congestion, infections) were observed between the regimens. Non-relapse mortality by day 30 post-transplant was 0% in both groups. A median follow-up was 29 (range: 1–119) months. The 3-yr DFS in BEAM and BEAC groups was 58% and 64%, respectively ( $P = .65$ ). There was no difference in the 3-yr OS between the groups (70% in BEAM and 84% in BEAC group;  $P = .51$ ). There was a trend to improved DFS and OS in patients transplanted in CR1 receiving BEAM ( $P = .09$ , Figure). In multivariate analysis, low-to-intermediate MIPI and transplant in CR1 were found to significantly increase PFS ( $P = .04$  and  $.01$ , respectively), while the HDT regimen did not affect PFS. BEAC and BEAM HDT regimens followed by ASCT had similar efficacy in MCL patients. There was a trend to improved DFS and OS in patients transplanted in CR1 and treated with BEAM vs BEAC. The toxicity profile was similar in both groups, except a significantly higher rate of grade 3–4 upper GI mucositis.

[P610]

Figure: Disease-free survival in patients transplanted in CR1



**Disclosure of conflict of interest:** None.

**P611**

**Efficacy of allogeneic hematopoietic cell transplantation in peripheral T-cell lymphomas in the front-line and relapsed/refractory setting: Results of a systematic review/meta-analysis**

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Peripheral T-cell lymphomas (PTCL) comprise a heterogeneous group of diseases among which PTCL-not otherwise specified (PTCL-NOS) represents the most common histology. Patients with PTCL are typically offered high-dose chemotherapy followed by autologous hematopoietic cell transplantation (auto-HCT) as front-line consolidation. Allogeneic HCT (allo-HCT) is generally offered in the relapsed setting; however, in selected cases it is also offered as front-line consolidation. No randomized controlled trial (RCT) have been performed to date comparing offering an allo-HCT versus other treatment modalities either in the front-line or in the relapsed setting. Thus, we performed this systematic review/meta-analysis to assess the totality of evidence pertaining to the role of allo-HCT in PTCL. Search of the literature was undertaken via PubMed and Web of Science from inception until September 6, 2016. No search limits were applied but studies presented only in abstract form were excluded. Data were collected on treatment benefits (complete remission (CR), progression-free survival (PFS), overall survival (OS)) and harms (non-relapse mortality (NRM), grade II-IV acute graft-versus-host disease (GVHD), and chronic GVHD). The search identified 1271 references; however, only 17 studies (6 in front-line ( $n=132$  pts), 11 in relapsed/refractory setting ( $n=330$  pts)) were eligible based on our inclusion criteria and had extractable data. Three studies included both frontline and relapsed/refractory cases but data for certain outcomes were reported separately. The median follow-up time for studies evaluating allo-HCT in the front-line or relapsed/refractory setting ranged from 30–45 months and 12–85 months, respectively. In the front-line setting, allo-HCT resulted in CR rates of 64% (95% CI = 50–77%), 2 studies,  $n=49$  pts), PFS rate of 64% (95% CI = 49–78%), 5 studies,  $n=100$  pts), and OS rate of 72% (95% CI = 62–81%), 5 studies,  $n=95$  pts). NRM rate was 6% (95% CI = 0–15%), 3 studies,  $n=68$  pts). Acute (grade II–IV) and chronic GVHD rates were 39% (95% CI = 24–56%), 2 studies,  $n=38$  pts) and 33% (95% CI = 16–53%), 3 studies,  $n=64$  pts), respectively. In the relapsed/refractory setting, allo-HCT resulted in CR rates of 68% (95% CI = 70–97%), 5 studies,  $n=75$  pts), PFS rate of 39% (95% CI = 31–47%), 6 studies,  $n=203$  pts), and OS rate of 52% (95% CI = 43–60%), 6 studies,  $n=307$  pts). NRM rate was 21% (95% CI = 13–31%), 7 studies,  $n=194$  pts). Acute (grade II–IV) and chronic GVHD rates were 34% (95% CI = 23–46%), 7 studies,  $n=197$  pts) and 37% (95% CI = 30–44%), 8 studies,  $n=199$  pts), respectively. Notwithstanding the need to perform a RCT to compare the efficacy of allo-HCT versus auto-HCT as front-line consolidation in PTCL, the results of this systematic review/meta-analysis show very encouraging OS rates of 72% following allo-HCT. Moreover, allo-HCT also offers an encouraging OS rate of 52% in patients with PTCL in the relapsed/refractory setting. The higher NRM rate in the relapsed/refractory setting probably reflects the adverse effect of a higher number of prior prescribed therapies. One of the limitations of our analysis is the inability to analyze outcomes for individual histologic subtypes due to the aggregate nature of the published data.

**Disclosure of conflict of interest:** None.

### P612

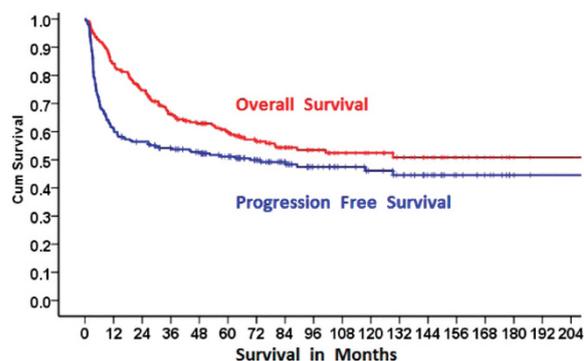
#### High-risk patients with relapse or refractory Hodgkin lymphoma do significantly better after HDC auto-SCT compared to control arm of AETHERA trial. Mature results from a cohort of 234 patients

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Brentuximab vedotin use in Hodgkin lymphoma (HL) patients who had HDC auto-SCT has been reported to improve progression free survival (PFS) but not the overall survival

(OS) in a phase 3 trial (Lancet 2015;385:1853-62). In this trial, after HDC auto-SCT, 329 high risk HL patients were randomized to receive placebo (control gp) vs brentuximab (experimental gp) as consolidation therapy. We are reporting our experience of patients with similar selection criteria as control gp. HL patients  $\geq 14$  yrs who received HDC auto-SCT with similar selection criteria as defined in AETHERA trial were identified that is, patients had at least one of the following risk factors for progression after HDC auto-SCT: (a) primary refractory HL (PR-HL), (b) relapsed HL with an initial remission duration of less than 12 months, or (c) extranodal involvement at the start of pre-transplantation salvage chemotherapy. At our institution, PR-HL is defined as partial response (PR), no response (NR), stable disease (SD), progressive disease (PD), relapsing within 3 months of finishing the planned treatment. Progression free (PFS) and overall survival (OS) from the day 0 of auto-SCT was estimated by Kaplan–Meier (KM) method. From 1996 to 2014, 234 patients with AETHERA trial criteria were identified. Male 121 (52%), female 113 (48%), median age at diagnosis: 22 yrs (12–61), at auto-SCT: 24.3 yrs (13.8–63) (90% < 40 yrs). Initial chemo: ABVD in 194 (83%). 70 (30%) had radiation therapy (XRT) after initial chemo. Response to initial chemo + XRT was refractory disease: 152 (65%), relapse between 3–12 months: 49 (21%) and relapse after 12 months: 33 (14%). Prior to salvage, extranodal disease: 138 (59%) and 48 (20%) had B symptoms. 177 (75.6%) received 1 line of salvage, 49 (21%) 2 lines and 8 (3.4%) had > 3 lines. ESHAP as first line salvage in 205 (87.6%). Before auto-SCT, response was CR: 74 (31.6%), PR: 146 (62.4%), SD/no response: 14 (6%). 140 (60%) patients had a FDG-PET scan post salvage; 55/140 (39%) with negative FDG-PET. BEAM used as HDC. Median follow-up is 83 months (15–227) from auto-SCT. Post auto-SCT response in 167 patients (71.4%); CR 148 (63%), PR 19 (8%), NR/SD 1 (0.5%), PD in 54 (23%), unknown 12 (5%). 35 (15%) had relapsed. At last follow-up in November 2016, 126 patients (54%) are alive with no disease, 6 (2.6%) alive with disease, 84 (36%) died of disease and 18 (8%) died of TRM or unrelated causes. For whole group, KM median PFS is 69 months (at 1,2,3,5,7 yrs  $\hat{a}$  61%, 56.4%, 54%, 51%, 48%). OS has not reached yet (at 1,2,3,5,7 yrs  $\hat{a}$  84%, 75%, 66%, 60%, 54%). We observed marked differences in patient characteristics between AETHERA and our report; mainly, half of AETHERA patients were heavily pretreated (> 2 lines of salvage) as compared to only 1/4<sup>th</sup> of our patients. In AETHERA, 1/3 received BEAM, we used BEAM 99%. AETHERA had 28% stable disease before SCT vs we have only 6%. AETHERA 24 months PFS (45% control arm, 65% brentuximab arm, investigator assessment) and our 56.4% is not much different. Despite having similar selection criteria, our median PFS is higher than both AETHERA trial placebo and experimental arm. Clinically, rate of progression in both studies are very high and comparable at 24 months. Given the very high cost of this drug and while waiting for survival benefit at this time, careful selection to identify a high risk group that may truly benefit from brentuximab is warranted.

[P612]



**Disclosure of conflict of interest:** None to disclose

**Disclosure of conflict of interest:** None.

**P613****High-dose melphalane and autologous peripheral blood stem cell transplantation as a salvage therapy for diffuse large B-cell lymphoma refractory to 2nd or 3rd line treatment**

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Early or refractory relapsed (< 1 year) diffuse large B-cell lymphoma has a very poor prognosis especially for those not responding to salvage chemotherapy. Allogeneic stem cell transplantation is potentially curative. Even though this is less likely in those not responding or having frank progression pre-transplantation. Methods: At our institution we identified all patients with aggressive B-cell lymphoma (diffuse-large B-cell lymphoma and blastoid mantle cell lymphoma) who were refractory or progressive to salvage chemotherapy with R-DHAP and who had peripheral blood stem cells ( $>2 \times 10^6$  CD34+/kg body weight) collected after the 1st or 2nd cycle. As a remission induction before allogeneic stem cell transplantation these patients received salvage high-dose melphalane and autologous stem cell transplantation. Allogeneic transplantation was performed from sibling donors or matched unrelated or mismatched unrelated donors: The conditioning regimen with fludarabine, busulfane, cyclophosphamide and anti-thymocyte globuline as published (Glass et al; Lancet Oncology 2014 Jun;15(7):757-66) was used in all except of 2 patients. Statistical analysis was performed with Prism; GraphPad Software; (La Jolla CA) Results: From 2006 to 2015 we identified 28 patients (21 male, 7 female, age 18–67 years, median age 47 years), 25 had diffuse large B-cell lymphoma and 3 had blastoid mantle cell lymphoma. 22 had relapsed within 1 year after primary diagnosis and 6 within a median of 25.3 months. After high-dose melphalane and autologous stem cell transplantation 13 patients had a partial and 6 a complete remission. 1 patient died due to neutropenic infection, 2 patients died due to progressive disease leading to a transplant related mortality of 3.5%. Median progression-free survival after autologous transplantation was 4.6 months. 24 proceeded to allogeneic stem cell transplantation. 8 patients had a matched related sibling, 9 had a matched unrelated donor and 7 had a mismatched unrelated donor. Transplant related mortality was 42% in this heavily pretreated population. 2-year overall survival of all patients intended for treatment is 21%. One of these patients with relapsed mediastinal lymphoma after allogeneic transplantation was cured by salvage radiotherapy and is in long-term remission (>2 years). Conclusions: Salvage high-dose melphalane and autologous peripheral blood stem cell transplantation for diffuse large B-cell lymphoma as a bridge to allogeneic transplantation is potentially curative for a minor fraction of these patients. However, the remission rate of 79% (46% PR, 21% CR) and progression-free survival of 4.6 months after high-dose melphalane and autologous stem cell transplantation provides a window of opportunity to use new drugs and cellular therapies in these poor prognosis patients.

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**Disclosure of conflict of interest:** None.

**P614****High-dose therapy and autologous hematopoietic progenitor cells transplantation for relapsed or refractory Hodgkin lymphoma: A follow up analysis of King Hussein cancer center results and prognostic variables**

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High dose chemotherapy and autologous stem cell transplantation is the treatment of choice for patients with relapsed refractory Hodgkin Lymphoma. Several factors including number of chemotherapy lines received before conditioning, time of relapse and remission status before transplantation can predict survival and PFS in patients undergoing autologous stem cell transplantation. In 2012, we reported on a 63 patients who underwent high dose chemotherapy followed by autologous stem cell transplantation from 2003 to 2008. All patients with relapsed or refractory Hodgkin Lymphoma in the period of 2009–2013, who underwent high dose chemotherapy followed by autologous transplantation were retrospectively analyzed. The main outcomes of the study were complete remission (CR) at day 100, overall survival (OS) and relapse-free survival (RFS). The impact of the following variables on OS and RFS: (a) disease status at the time of transplant, (b) number of chemotherapy lines prior to conditioning and (c) time of relapse 12 months and (d) age. A total of 78 patients were identified. The median age was 31 year. There were 50.6% females and 49.4% males. Complete remission (CR) was achieved in 48.7% of patients and 49.9% with chemotherapy sensitive disease at the time of transplantation. Prior to conditioning regimen, 43.2% received two chemotherapy lines, and 56.8% received more than two lines. 41% relapsed in less than 12 months and 57% relapsed more than 12 months after completion of therapy CR at day 100 was 69.2%. The median OS for the whole group was 62.0 months; the median RFS was 10.6 months. The number of chemotherapy lines significantly impacted OS and EFS. CR status before conditioning, favorably influenced OS and EFS with a trend toward better OS in favor of those who underwent ABMT while in complete remission. The time of relapse and the age did not affect survival outcomes.

[P614]

Name	Value	Number (%)
Gender	F	39 (50.6%)
	M	38 (49.4.5.0)
Age	Adults	78 (73.6%)
	Pediatrics	28 (26.4%)
Remission	CR	38 (48.7%)
	DP	1 (1.3%)
	NA	3 (3.8%)
	PR	20 (25.6)
Number of chem. lines	SD	16 (20.5%)
	=<2	32 (43.2%)
Time of relapse	>2	42 (56.8%)
	<12 months	32 (41%)
	>12 months	45 (57%)

The outcome of patients with relapsed or refractory Hodgkin Lymphoma is favorable and the number of chemotherapy lines received before conditioning is the only factor that had a statistically significant impact on OS and EFS.

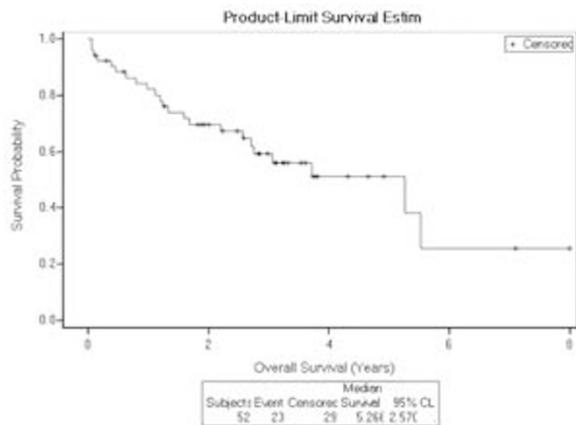
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[P614]



**Disclosure of conflict of interest:** None.

**P615**  
**HIV-related hematological malignancies: A single-center experience**

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Since the identification of Human Immunodeficiency Virus (HIV), a clear association between HIV and specific malignancies has been recognized. High-grade B cell lymphomas are the most common malignancy complicating HIV infection and one of three AIDS defining malignancies. Diffuse large B cell lymphoma (DLBCL) accounts for 80% of cases. Before 1996, lymphomas were the cause of 16% of all deaths

attributable to AIDS. After the introduction of highly active antiretroviral therapy (HAART) overall incidence of ADM declined, however longer survival and exposure to environmental risk factors have increased the incidence of non ADM (ADM) such as Hodgkin's lymphoma (HL). Since HAART has improved overall survival substantially, the aim of chemotherapy should be complete remission rather than palliation with careful consideration of drug interactions and side of HAART. Between 2011 and 2016 a total of 510 patients were detected HIV positive. Twenty-one of these patients were diagnosed with a malignancy and 8 patients referred to our department with a hematologic malignancy were evaluated retrospectively. Diagnosis, stage, treatment, survival data were recorded. HAART during chemotherapy, nadir CD4 count and CD4 count at diagnosis of malignancy was evaluated. Four patients were diagnosed with high grade B cell lymphoma, 2 patients with primary central nervous system lymphoma (PCNSL), 1 patient with HL and 1 patient with multiple myeloma (MM). All patients were male and median age at diagnosis was 40.5 (24–63). HIV seropositivity was identified during evaluation of malignancy in both PCNSL patients. Median duration of HIV seropositivity before diagnosis of malignancy was 18 months (8–42) for the remaining patients. Patient characteristics, treatment modification and CD4 counts are summarized in Tables 1 and 2. Lymphoma was fatal in 5 and the cause of death was identified as lymphoma progression in all patients including one patient diagnosed with Hodgkin's Lymphoma. A patient presented with multiple plasmocytomas was diagnosed with multiple myeloma is currently receiving induction treatment together with HAART. HIV related lymphoma patients frequently present with extra nodal disease, incidence of central nervous system involvement is also higher and prognostic score tends to be in the intermediate or high-risk groups. Prognosis is also worse than HIV negative population. Degree of immunosuppression is implicated and the duration of immunosuppression is directly correlated with the risk of developing lymphoma rather than HIV itself. HAART allowed the use of aggressive chemotherapy since it improved immune system and decreased infectious complications. Multiple myeloma is a rare neoplasm observed in HIV infection and the treatment is based on data obtained from HIV negative patients. Treatment of such patients as well as lymphomas should take into consideration the toxic effects of HAART combined with chemotherapy. Since HIV positive

[P615]

	Sex	Age at Diagnosis	Diagnosis	Duration of HIV diagnosis before malignancy (months)	Stage	Systemic Chemotherapy (cycles)	Survival after diagnosis of malignancy (months)
Patient 1	Male	28	BL	15	IVB	4	8
Patient 2	Male	63	DLBCL	15	IVB	3	6
Patient 3	Male	40	PCNSL	0	NA	1	2
Patient 4	Male	24	PCNSL	0	NA	2	14
Patient 5	Male	51	DLBCL	0	IIB	0	1
Patient 6	Male	33	DLBCL	42	IVB	0	NA
<b>Others</b>							
Patient 1	Male	34	HL	8	IVB	6	10
Patient 2	Male	51	MM	18	I	2	2

BL:Burkitt's Lymphoma, DLBCL: Diffuse Large B Cell Lymphoma, PCNSL: Primary Central Nervous System Lymphoma, HL: Hodgkin's Lymphoma, MM: Multiple Myeloma, NA: Not applicable

patients are excluded from most studies, there are no guidelines to direct treatment and avoid toxicities. Drug interactions should be monitored closely and modifications should be made accordingly. Interruption of HAART may not be mandatory since studies have shown safety of continuation of HAART during chemotherapy. For newly diagnosed HIV and malignancy, careful clinical and laboratory evaluation should be made before postponing HAART until after chemotherapy.

**Disclosure of conflict of interest:** None.

#### P616

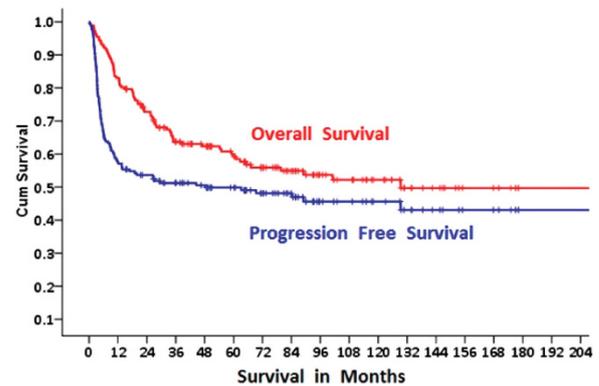
### Long term survival of refractory Hodgkin lymphoma after high dose chemotherapy (HDC) and autologous stem cell transplant (ASCT). Single institution analysis of 177 patients

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The outcome of HDCT and ASCT in refractory Hodgkin lymphoma (R-HL) is not as encouraging as in relapsed HL. Ten years ago we analyzed and reported outcomes of ASCT in our R-HL patients, however the follow-up was short. Now we are reporting long term outcomes in R-HL after ASCT in one of the largest numbers reported to date. Between 1996 and 2014, patients with HL who underwent HDC and ASCT for R-HL in Adult Medical Oncology (age > 14 years) were identified. R-HL is defined as partial response (PR), no response (NR), stable disease (SD), progressive disease (PD), relapsing within 3 months (relapse < 3 m) of finishing the planned (chemotherapy + radiation therapy (XRT)) treatment or refractory to salvage chemotherapy. Kaplan–Meier (KM) method was used to estimate progression free survival (PFS) and overall survival (OS) from the day 0 of ASCT while progression is defined as progression of disease, relapse and death from any cause. All percentages are rounded to nearest. 307 patients underwent HDC and ASCT during 1996–2014 and 177 of them met the criteria of R-HL. Male 97 (55%), female 80 (45%), Median age at diagnosis was 22.2 years (8–61 years) and at ASCT was 24 years (14–62 years). Initial therapy was ABVD in 153 (86.4%), MOPP/COPP alternating with ABV or ABVD in 11 (6%) and others in 13 (7%). 49 (28%) had XRT after initial chemotherapy. Response to initial chemo + XRT was PR in 80 (45%), PD in 51 (29%), CR in 38 (21%) (28/38 relapsed within 3 months and others have refractory relapse) and no response in 4 (2%) and others in 4 (2%). Prior to salvage chemotherapy, 119 (67%) had stage III–IV, 90 (51%) extra-nodal involvement, 43(24%) bulky disease and 31 (18%) had B symptoms, spleen involvement in 43(24%), performance status 0, 1 in 155 (88%). ESHAP was used as first line salvage in 153 (86%) or 3<sup>rd</sup> line 13 (7%). Post salvage / prior to HDC and ASCT disease status was PR in 112 (63%), CR in 53 (30%) and NR/SD in 12 (7%). 111 (63%) patients had a FDG-PET scan prior to ASCT, 45 (25%) were in CR. BEAM was used as conditioning regimen. Median follow-up for all alive patients is 81 months (15–224) from ASCT. Response rate post ASCT: CR in 105 (59.3%), PR in 16 (9%), NR/SD in 1 (0.6%) and PD in 45 (25.4%) patients, others /unknown in 10 (5.6%). 61 (35%) patients had XRT post auto-SCT. Type of first post HDC auto-SCT event was no event in 81 (46%), persistent disease in 17 (10%), PD in 45 (25%), relapsed disease in 23 (13%), treatment related mortality in 6 (3%) and died of other cause 5 (3%). At last follow-up in November 2016, 94 patients (53%) are alive with no disease, 6 (3%) alive with disease, 64 (36%) died of disease and 13 (7%) died of treatment related mortality or other causes. For entire group, KM estimated median OS is 129 months, 1,2,3,5 and 7 year survival is 83%:73%:64%:59%:55% respectively. Median PFS is 48.5 month, 1,2,3,5 and 7 year PFS is 57.6%:53.6%:51%:50%:47% respectively. We are reporting a very high risk group of patients with a very long follow-up. In patients with R-HL, ESHAP + BEAM combination resulted in high response rate (68.3%). These remissions are durable. A 5 year OS survival of greater than 50% in our population is

higher than most reports with similar numbers. Although our cohort has a 7 year OS survival of 55%, 45% patients have either relapsed or died underscoring need for improvements in the management refractory HL.

[P616]



**Disclosure of conflict of interest:** None.

#### P617

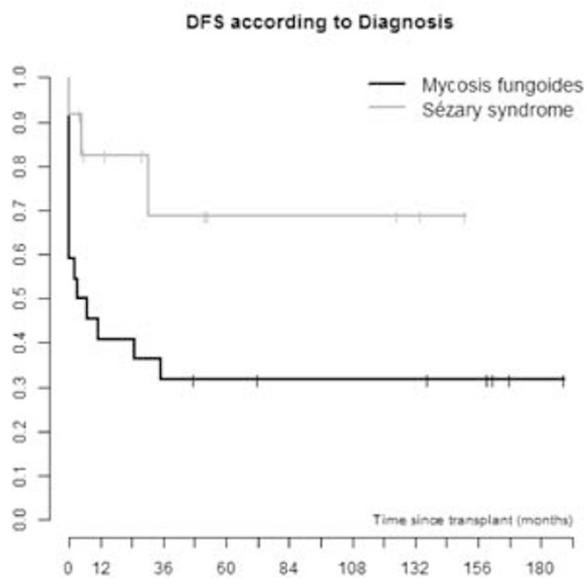
### Long-term outcome of reduced intensity conditioning allogeneic stem cell transplantation in advanced stage mycosis fungoides and Sézary syndrome

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Although over the last decade a few novel compounds have been shown promising activity in mycosis fungoides (MF) and Sézary syndrome (SS), life expectation of patients with advanced stages or refractory disease still remain very poor, with median survival ranging from 1.4 to 3.4 years (Agar NS et al. JCO 2010). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents the only current curative strategy in selected patients with CTCL. Here we update the previously reported results of our reduced-intensity conditioning (RIC) allo-HSCT experimental program, initiated in 2002. As of November 2016, in our Centre 34 patients underwent RIC allo-HSCT. Donors were HLA-identical sibling in 16, fully-matched unrelated in 7, 1 or 2-mismatch-unrelated in 9 and haploidentical relative in 2. Median age was 53 years (range: 19–66). All patients (22 M and 12 F) had stage IIB/IV refractory MF (n=22) or refractory SS (n=12). Median number of previous treatment lines was 6 (range: 2–12). Source of stem cells was peripheral blood in 31 patients and bone marrow in 3. Median time from diagnosis to HSCT was 46 months (range: 13–264). Conditioning included Flu/CTX/TBI200, pentostatin +TBI200 and Flu/Mel in case of HLA-identical or unrelated donor, whereas the TT/Flu/CTX/TBI200 regimen was used in the haplo setting. GvHD prophylaxis included CsA/MMF in all patients, with the addition of ATG in cases with unrelated donor and post-transplant CTX (50 mg/kg giorni +3 e +4) in cases with haploidentical donor. Full donor chimerism was obtained in 28/33 of the evaluable patients, in a median time of 2 months (range: 1–12). Grade II–IV acute GvHD occurred in 16 patients (57%), while grade III–IV was observed in 8 patients (28%). Chronic GvHD occurred in 10 patients (36%), being extensive in 4 (14%), all transplanted from HLA-identical sibling (no ATG). Following transplantation, a complete remission (CR) was achieved in 22 out of the 33 evaluable patients (67%), of whom 2 experienced relapse at +25 and +35 months, respectively. Transplant-related death occurred in

6 patients (17%), of whom 4 were in CR. Out of the 11 patients who did not achieve CR, 9 died from progressive disease (median follow-up of 12 months, range: 3–31), 1 from a secondary malignancy and 1 is still alive with disease 41 months after transplant. Of note, all pts who died in progression had chemoresistant disease at time of transplant. At the last follow-up, 18 patients were alive and 16 (89%) maintained CR after a median time of 66 months (range: 4–189). In the whole population, the 5-year overall survival was 52% (95% CI 34–70) and the 5-year disease-free survival (DFS) was 44% (95% CI 27–62). However, when MF and SS were analysed separately, 5-yrs DFS were 32% (95% CI 12–51) and 69% (95% CI 38–99), respectively (Figure). Apart from diagnosis, outcome appeared to be primarily associated with the disease status at transplantation, with a 5-yr DFS of 100% in the group of patients ( $n = 8$ ) who were in CR before starting the conditioning. After a median follow-up longer than 5 years, we confirm the efficacy of RIC allo-HSCT as a powerful therapeutic strategy in inducing and maintaining remission in selected patients with chemosensitive advanced-stage CTCL, with results particularly encouraging in SS.

[P617]



**Disclosure of conflict of interest:** None.

**P618**

**Outcomes of allogeneic hematopoietic stem cell transplantation for Hodgkin lymphomas: A retrospective multicenter experience of the rete ematologica pugliese (REP)**

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Hodgkin's lymphoma (HL) is a potentially curable disease, and modern therapy is expected to successfully cure more than 80% of the patients. Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have an established role in the management of refractory and relapsed HL, leading to long-lasting responses in approximately 50% of relapsed patients and a minority of refractory patients. Patients progressing after intensive treatments, such as autologous SCT, have a very poor outcome. Allogeneic SCT

represents the only strategy with a curative potential for these patients; This study reports a retrospective multicenter experience of the Rete Ematologica Pugliese (REP) over the past 16 years aiming to define the impact of patient, disease, and transplant-related characteristics on outcomes. 67 patients with histologically confirmed diagnosis of HL who received allogeneic SCT from 2000 to 2016 were retrospectively studied. The median age was 34 years (range: 16–57 years) and 36 (54%) were male. The majority of patients (84%) had had a prior autologous SCT. At the time of allogeneic SCT, 28 (42%) patients had a chemosensitive disease and 39 (58%) were chemorefractory. Most (93%) patients received reduced-intensity conditioning, 54% received matched sibling donor and 46% matched-unrelated donor grafts. The disease status at day 100 post-transplant was reported in 62 out of 67 evaluable patients. Of the 26 patients with chemosensitive disease, 18 (70%) achieved a CR, 7 (27%) had a PR or stable disease and 1 (3%) had progressive disease. Of the 36 patients with chemorefractory disease 7 achieved a CR (20%), 26 had a PR or stable disease (72%) and 3 (8%) had progressive disease. Following transplantation 40 patients have relapsed or progressed at a median time of 6.3 months (range: 1–59 months) post-transplant. With a median follow-up of 38 months (range: 3–195 months) 41 patients remain alive and 26 have died. The Kaplan–Meier estimates of OS and PFS at five years were 41% and 35% respectively. 11 patients (16%) died of non-relapse mortality at a median of 300 days (range: 28 days– 40 months) following transplantation. The causes of death included infection ( $n = 6$ ), GVHD ( $n = 3$ ), multi-organ failure ( $n = 2$ ). Allogeneic SCT results extend survival in selected patients with relapsed/refractory HL with low treatment-related mortality. Patients who have active disease at the time of allogeneic transplantation have poor outcomes. Allogeneic SCT may be an effective salvage strategy for patients who relapse after an autologous SCT.

**Disclosure of conflict of interest:** None.

**P619**

**Outcomes of both high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation and conventional chemotherapy alone for mantle cell lymphoma: A 12 -year single-center experience**

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Although the overall survival has improved significantly in mantle cell lymphoma (MCL) according to advanced treatment options, relapsed or refractory disease remains a challenge. Recently, lots of targeted agents actively have been tried clinical studies and adapted to clinical practice in indolent lymphoma. However, the role of frontline autologous hematopoietic stem cell transplantation (auto-HSCT) has not been fully understood in patients with MCL, compared with a few impressive published data about auto-HSCT as salvage treatment option for patients with relapsed MCL. So, we retrospectively evaluated consecutive patients diagnosed MCL, and compared the clinical outcomes of high-dose chemotherapy followed by auto-HSCT and conventional chemotherapy alone. Between January 2003 and December 2014, consecutive patients with newly diagnosed with MCL at Catholic Blood and Marrow Transplantation Center in South Korea were included in this study. All of the patients received high-dose cytarabine-containing regimen or CHOP with/without rituximab regimen for induction therapy regardless of transplant eligibility. The treatment approach in our institution for patients was based on the physician discretion for transplant eligibility or ineligibility that depend on patient age, comorbidities, and disease status. Seventy patients were included in the analysis. Initial chemotherapy regimens were consisted of CHOP ( $n = 12$ , 17%), R-CHOP ( $n = 44$ , 63%), R-hyperCVAD ( $n = 10$ , 14%), and hyperCAVD ( $n = 4$ , 6%). Demographics and disease characteristics of both groups are shown in Table1. Patients received auto-HSCT were superior

overall survival (OS;  $P=0.015$ ) and progression-free survival (PFS;  $P < 0.001$ ). The subgroup analysis according to high-risk of MCL international prognostic index (MIPI) or bone marrow involvement was performed. Between the two treatment arms among the high-risk MCL group, the clinical parameters were not different. The high-risk MCL patients with frontline auto-HSCT showed superior OS ( $P=0.0216$ ) and PFS ( $P < 0.001$ ) compared with conventional chemotherapy alone. Although MCL is classified within indolent lymphoma, frontline auto-HSCT can be considered for patients diagnosed with MCL in the group of high-risk MIPI or BM involvement with the favorable survival outcomes.

**Disclosure of conflict of interest:** None.

**P620**

**Outcomes of hematopoietic stem cell transplant for extranodal natural killer/T-cell lymphoma, nasal type: A study from the Société Francophone de Greffe de Moelle et de Thérapie cellulaire**

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Nasal type extranodal NK/T-cell lymphoma (ENKTL) is a very rare and aggressive malignancy characterized by a poor outcome. Current standard therapy is not yet established. The role of high dose therapy followed by haematopoietic stem cell transplantation (HSCT) is still controversial. We evaluated the outcomes of all the ENKTL patients undergoing HSCT in a multicenter analysis on patients registered by the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) and compared them with a population of French patients who received chemotherapy alone. Sixty four ENKTL (48 males and 16 females) received HSCT, including 19 allogeneic (alloSCT) and 45 autologous transplantations (autoSCT). Median age at the time of HSCT was 43 years (range: 17 to 70 years). Overall, 57% of the patients presented with disseminated disease (64% and 55% in the alloSCT and autoSCT, respectively), 61% were in complete response (CR) at the time of HSCT (74% and 61% in alloSCT and autoSCT groups, respectively) and 82% had received L-asparaginase regimen prior to HSCT (73% and 84% in alloSCT and autoSCT groups, respectively). Five (26%) and 20 (44%) patients of the alloSCT and autoSCT groups underwent upfront HSCT therapy, respectively. Four patients received tandem autologous/allogeneic transplants. In alloSCT, stem cell source was a matched related donor in 13 patients, an unrelated donor in 3 patients and an umbilical cord blood in 3 patients. Reduced intensity conditioning regimens (based on Fludarabine-Busulfan combination) and BEAM regimen were used in 42% and 84% of patients from the alloSCT and autoSCT groups, respectively. Median overall survival for the whole cohort was 17.1 months (range: 1 to 131 months). The 3-year non-relapse mortality was 16.1% and 22.7% in the alloSCT and autoSCT groups, respectively ( $P=0.58$ ). The 3-year overall survival (OS) and progression free survival (PFS) were 47.5% and 40.8% in the autoSCT and 43.4% and 47.4% in the alloSCT group,

[P620]

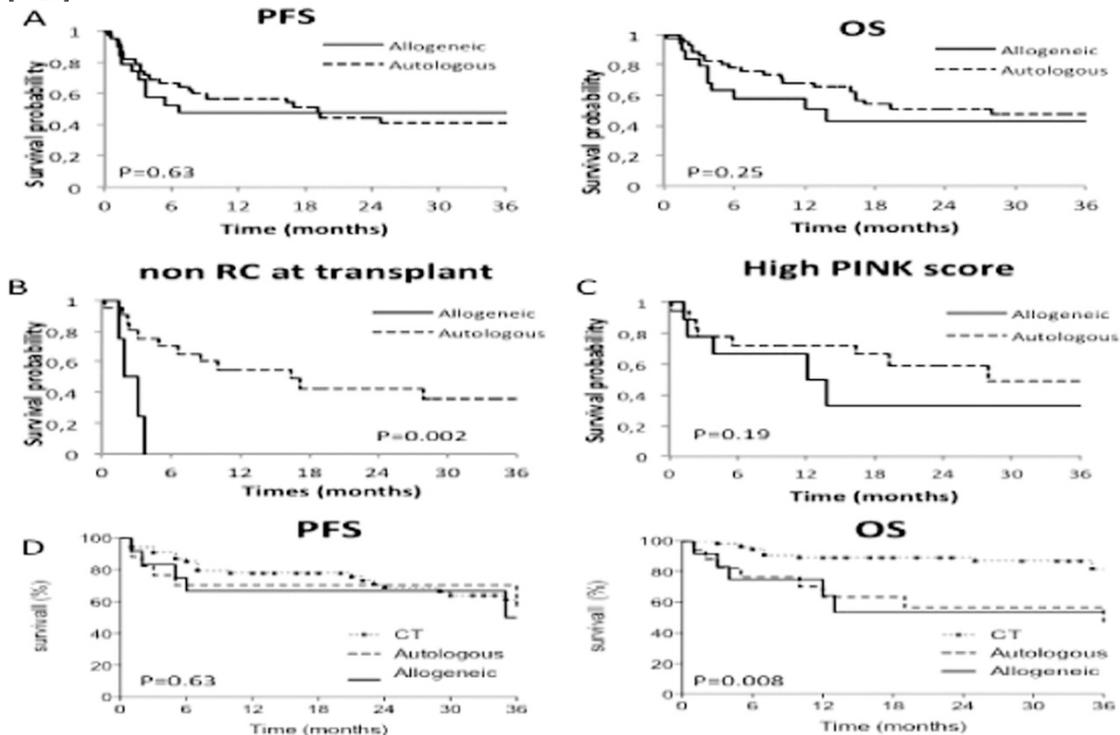


Figure 1. Progression free survival (PFS) and overall survival (OS) according to the transplant group (A). Overall survival in patients transplanted non complete remission (CR) at transplant (B) with high PINK risk score at diagnosis (C) according to the transplant group. Overall survival in patients with RC1 (D) according to transplant group (n=17 autologous and n= 12 allogeneic) and chemotherapy alone (CT) (n=55) group. Solid line: allogeneic group; dotted line: autologous group; dotted line with square symbol: chemotherapy alone.

respectively (Figure 1A). The absence of CR prior to HSCT was associated with a poor prognosis ( $P=0.008$ ). As compared to alloSCT, autoSCT resulted in a better outcome in patients who didn't achieve CR before transplant ( $P=0.002$ ) and tended to have better outcome in high PINK risk score (Figure 1 B–C). Finally, at 3 years PFS and OS of patients who have been treated by chemotherapy alone (CT) ( $n=55$ ) or followed by alloSCT ( $n=12$ ) or autoSCT ( $n=17$ ) in CR1 were 55%, 81% and 50%, 54% and 44%, 50%, respectively (Figure 1D). In this French cohort, more patients received autologous HSCT in upfront therapy than allogeneic HSCT. In CR1, there is no evidence suggesting that transplantation is associated to a better outcome than chemotherapy alone. However, a precise matching based on the PINK score will be evaluated to ensure that patients who were intensified were not of worst prognosis. In refractory patients there is also no clear advantage to perform alloSCT when compared to autoSCT. However, in relapsing disease after CT or AutoSCT alloSCT, allowed to obtained durable control of the disease.

**Disclosure of conflict of interest:** None.

## P621

### Phase I study of myeloablative conditioning regimen of full-dose busulfan, melphalan, and fludarabine for allogeneic hematopoietic stem cell transplantation in patients with refractory or relapsed aggressive lymphoma

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High relapse rate is one of concerns for allo-SCT in pts with relapsed/refractory aggressive lymphoma. An optimal conditioning regimen designed for aggressive lymphoma may reduce relapse, especially during early post-transplantation period. However, it is not established yet. Results of a German phase 2 study of allo-SCT with conditioning regimen of fludarabine, busulfan (12 mg/kg po or 9.6 mg/kg iv), and cyclophosphamide with or without post-transplantation rituximab for relapsed/refractory aggressive lymphoma suggested the role of myeloablative busulfan-containing regimen in reducing relapse rate in pts with aggressive lymphoma. Based on these results, we conducted a single institution prospective study to explore feasibility of the BMF regimen consisted of full-dose busulfan, melphalan, and fludarabine in pts with relapsed/refractory aggressive lymphoma (UMIN00013940). Patients with aggressive lymphoma who achieved at least SD with salvage chemotherapy after experiencing either PD during first-line therapy, early relapse (< 12 mo) after first-line therapy, late relapse ( $\geq 12$  mo) but refractory to salvage therapy, relapse after auto-SCT,; age 20–65; ECOG PS of 0-2; and without severe organ dysfunction were eligible. Donor source could be 6/6 matched related or unrelated donor PB/BM or CB with  $\leq 2$  antigen mismatch; The BFM regimen was consisted of busulfan 12.8 mg/kg iv, fludarabine 180 mg/m<sup>2</sup>, and melphalan 80 mg/m<sup>2</sup> (Yamamoto H. BBMT 2016). GVHD prophylaxis was CsA + MTX (related PB), Tac + MTX (unrelated BM), and Tac + MMF (CB). Primary end point of the study was survival with engraftment at day 60, and secondary end points were engraftment rate at day 100; NRM and relapse rate at day 100 and 1 y; progression free survival (PFS), overall survival (OS), and GVHD at 1 y. Protocol was approved by IRB and written IC was obtained from all pts. Twelve pts (male 10, female 2) with a median age of 55 y (33–63) were enrolled. PS was 0–1 in 11 pts. Diagnosis were DLBCL ( $n=4$ ), transformed FL ( $n=3$ ), ENKTCL ( $n=3$ ), PTCL ( $n=1$ ), and AITL ( $n=1$ ). Median number of previous line of therapy was 3.5 (2–5) and 5 pts had failed previous auto-SCT. Diseases status at transplantation was CR ( $n=6$ ), PR ( $n=4$ ), and SD ( $n=2$ ). Donor source was CB ( $n=6$ ), unrelated BM ( $n=5$ ), and related PB ( $n=1$ ). Survival with engraftment at day 60, primary

endpoint of the study, was achieved in 100%. Neutrophil engraftment was achieved at a median of day 18 (13–32). Full donor chimerism at day 30 was achieved in all of the 11 pts evaluated. Two pts developed VOD which was manageable. With a median follow-up of 20 mo, 3 pts had progression of lymphoma at 2, 5, 6 mo. Five pts died and cause of death were progression of lymphoma in 3, interstitial pneumonitis in 1 (at 5 mo), systemic adenovirus infection in 1 (at 5 mo), and aGVHD in 1 (at 4 mo). OS and PFS at 1y were 54% and 46%, respectively. Relapse and NRM rates were 8% and 0% (day 100), and 27% and 27% (1y), respectively. aGVHD of grade II–IV was observed in 7/12 pts and 2 pts developed limited cGVHD. This prospective study shows that allo-SCT using myeloablative conditioning regimen with full-dose busulfan, melphalan, and fludarabine for relapsed/refractory aggressive lymphoma is feasible and deserves further evaluation.

**Disclosure of conflict of interest:** None.

## P622

### Primary cutaneous T cell lymphoma (CTCL): Long term follow-up after allogeneic hematopoietic stem cell transplant (HSCT)

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For patients with advanced CTCL, the allogeneic HSCT seems to be curative with graft versus lymphoma effect playing a major therapeutical role. In this retrospective study, 16 patients with a median age of 52 years (range: 24–67) affected by CTCL underwent allogeneic HSCT after a median of 4 (range: 1–8) lines of chemotherapy, including autologous transplant for 2 of them. The median time from diagnosis to HSCT was 46 months (range: 9–309). The diagnoses were: Sezary syndrome (SS,  $n=8$ ). Mycosis Fungoides (MF,  $n=2$ ), primary cutaneous CD30+ lymphoma ( $n=4$ ), panniculitis-like T-cell lymphoma ( $n=1$ ), NK T cell lymphoma ( $n=1$ ). At time of HSCT, 2 patients (12.5%) were in complete remission (CR), 9 (56.3%) in partial remission (PR) and 5 (31.2%) had active disease. The patients were transplanted from an HLA-identical ( $n=7$ ), mismatched ( $n=1$ ) or haploidentical ( $n=1$ ) sibling, from matched unrelated donor ( $n=5$ ) or from a single cord blood unit ( $n=2$ ). Different pre-transplant regimens were used as myeloablative (MAC) in 6 (Th-Bu-Flu,  $n=4$ ; Bu-Cy,  $n=2$ ) or as reduced intensity (RIC) in 10 (Th-Flu-Cy,  $n=7$ ; Th-Bu-Flu,  $n=3$ ). All patients engrafted for neutrophils at a median of 17 days (range: 12–46) and 14 patients engrafted for platelets at a median of 14 days (range: 12–77). Acute GVHD was of grade 0–I in 9 patients and II–IV in 7 (40.7%). Skin was the most common organ involved. Five of 11 evaluable patients experienced chronic GVHD which was mild in 3 and severe in 2. At a median of 3 months (range: 1–11), 7 patients died (4 MAC and 3 RIC) because of GVHD ( $n=4$ ), VOD ( $n=1$ ), pneumonia ( $n=1$ ) or multiorgan failure ( $n=1$ ). All 12 patients surviving at 3 months from transplant were in CR. Only patients prepared with a RIC ( $n=4$ ) relapsed respectively at 3, 9, 10 and 11 months from HSCT. These patients received DLI associated or not to chemotherapy. Three achieved CR, which remained stable in 2, while one patient died in CR from post DLI acute GVHD. One patient (NK-T cell) not achieving CR is still alive with active disease. For all 16 patients the median survival was 10 months (range 1–130). With a median follow up of 76 months (range: 4–130), 9 patients (2 MAC, 7 RIC) are alive, 8 in CR and 1 with active disease. At 10 years, the OS was  $54 \pm 13\%$ ; at 5 years DFS was  $34 \pm 12\%$ . According with the median time (46 months) from diagnosis to transplant, the 10-year OS was  $88 \pm 12\%$  for patients transplanted early and  $25 \pm 15\%$  for the others ( $P < 0.04$ ), while DFS was respectively

58 ± 19% and 13 ± 12% ( $P < 0.05$ ). Despite the small number of patients, our results confirm the high susceptibility of CTCL to the graft versus lymphoma effect and point out the time to transplant as a crucial prognostic factors for the outcome. Finally, the long-term follow up of our series strongly supports HSCT for the cure of CTCL.

**Disclosure of conflict of interest:** None.

#### P623

##### Results of frontline high dose chemotherapy in high-risk DLBCL patients stratified according to the NCCN-IPI

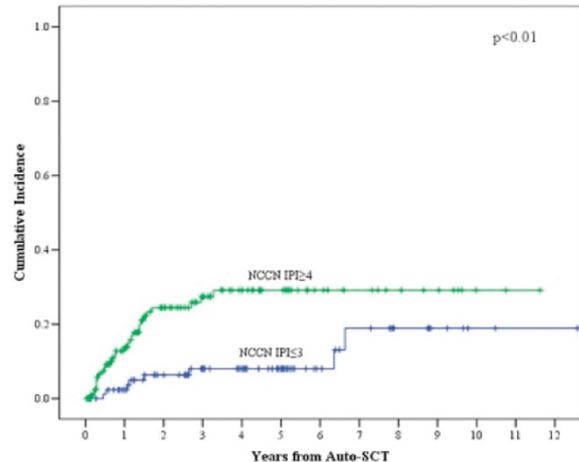
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Recently, a new prognostic score, the NCCN-International Prognostic Index (IPI) has been developed<sup>1</sup> to stratify patients affected by Diffuse Large B Cell Lymphoma (DLBCL), and in high-intermediate and high risk groups the survival was equal or less than 50%. The aim of this analysis was to evaluate the outcome of a cohort of DLBCL patients undergoing high dose chemotherapy (HDC) as consolidation following first line chemo-immunotherapy, after their re-classification according to the NCCN-IPI. We performed a retrospective study on 221 patients diagnosed with DLBCL, with a high/intermediate or high-risk disease according to the IPI (2–5), who received upfront HDC with ASCT, in 2 institutions. The patients were then re-stratified according to the NCCN-IPI and arbitrarily classified in 2 groups: low risk (NCCN-IPI ≤ 3) and high risk (NCCN-IPI ≥ 4). The pre-transplantation disease status was assessed by positron emission tomography (PET) or computed tomography (CT). The primary endpoints were non-relapse mortality, progression-free survival (PFS), overall survival (OS) and relapse risk. The estimated 3-year PFS for all patients was 80.1% (95% confidence interval [CI] 74.2–86.0) and the 3-year OS was 91.0% (95% CI 86.7–95.3). Of these patients, 93 had a low risk IPI score (IPI = 2) and 128 were considered high risk (IPI ≥ 3). Subsequently, the whole cohort was re-stratified according to the NCCN-IPI: 133 patients were allocated to the high-risk (NCCN-IPI ≥ 4) group, and 88 to the low-risk group (NCCN-IPI ≤ 3). The analyses were then carried out for both groups. The 3-year PFS was 92.0% (95% CI 85.7–98.3) in the low-risk group and 71.2% (95% CI, 62.2–80.2) in the high-risk group ( $P < 0.01$ ), whereas the 3-year OS was 98.8% (95% CI 96.4–100) in the low-risk group and 85.0% (95% CI 77.9–92.1) in the high-risk group ( $P = 0.02$ ). The significant difference in OS and PFS between the two groups was mainly due to the cumulative incidence of relapse at 3 years (graph 1): 8.0% (95% CI 3.2–15.7) in the low-risk group and 27.1% (95% CI 18.7–36.2) in the high-risk group ( $P < 0.01$ ). Non-relapse mortality was comparable in both cohorts: 1% (95% CI 0.2–3.3) for all patients. Figure 1: Cumulative incidence of relapse following HDC and according to NCCN-IPI. Patients affected by high-risk DLBCL still have an unsatisfactory prognosis after treatment with conventional therapy regimens, even in the Rituximab era. The 5-year OS and PFS in patients with NCCN-IPI score ≥ 4 range: from 33% to 64% and from 30% to 51% respectively<sup>1</sup>. Although this is a retrospective analysis subject to all related biases, our results suggest that upfront intensive therapy with autologous stem cell transplantation may significantly improve the outcome of these patients compared to conventional chemotherapy. The role of HDC in the treatment of DLBCL is controversial. However, new entities or new risk stratifications, as the one reported here, could allow to identify high risk subpopulations that could benefit from this approach.

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**Disclosure of conflict of interest:** None.

#### P624

##### Salvage treatment of relapsed enteropathy-associated/monomorphic epitheliotropic intestinal T-cell lymphoma using allogeneic hematopoietic cell transplantation (HCT)

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Enteropathy-associated T-cell lymphoma (EATL) is an exceedingly rare and often rapidly fatal subtype of peripheral T-cell lymphoma, arising from intraepithelial lymphocytes. EATL type I is associated with celiac disease; type II occurs in patients without inflammatory pre-conditions (according to WHO 2016 classification now called monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL)). Surgical debulking and anthracycline-based chemotherapy (CTx) followed by high-dose chemotherapy (HDCTx) and autologous cell rescue (ASCT) are pursued when possible in this often malnourished and frail patient cohort. Yet, even with intensive consolidation relapse occurs in 40–70% of patients. The value of allogeneic hematopoietic cell transplantation (HCT) is not clarified as of today due to limited reports. Here, we report on a patient with MEITL who was rescued with an allo-HCT for his 2nd relapse following prior ASCT. Moreover, we summarize the available literature on the use and outcomes of allo-HCT for EATL and MEITL. A 48y old man with spontaneous intestinal perforation was diagnosed with MEITL following emergency partial resection of the small intestine. Histology revealed infiltration by monotonous medium-sized lymphocytes with abnormal immunophenotype (CD3+, CD56+, CD8+, CD5-, CD30-, TIA-1+) consistent with type II EATL. Post-surgical 18F-FDG PET-CT scan showed abnormal uptake in gastric antrum and pyloric region but no other manifestations. CTx with CHO(E)P (6x) followed by BEAM HDCTx and ASCT was performed and achieved a complete remission (CR1). However, 9m post ASCT disease relapsed and was treated with 2x DHAP, and 4x DHAOx. CR2 was achieved after the 3rd cycle of salvage therapy. Due to anthracycline-induced cardiopathy allo-HCT could not be performed at that time. 5m after completion of salvage therapy, disease relapsed again, and was progressive under Pralatrexat treatment (1 cycle, 6 infusions). By then cardiac function had recovered and therapy was switched to dose-reduced mini-BEAM (2x). In CR3 reduced intensity conditioning (RIC; fludarabine, busulfan, ATG) and allogeneic HCT from a matched sibling donor was performed. Cyclosporin A (CsA) and Mycophenolate Mofetil (MMF) were given as GVHD prophylaxis. Prompt engraftment in blood (day+14) and full donor chimerism in the marrow (d+100)

**Table 1. Series of allogeneic HCT in EATL / MEITL**

Author (year)	EATL-type	n (EATL pt given allo-HCT)/ n (total EATL cohort)	Pretreatment	Prior ASCT	Remission at allo-HCT	Conditioning regimen, graft type	Relapse (alive vs. time-point of death)
Regelink et al. (2010)	I	2 of 2	surgery + 6 # CHOP	no	CR	RIC (Flu/Cy)	2/2 (died at 3m and 9w post-HCT)
Tse et al. (2012)	II	1 of 38	NA	no	PR	RIC (Flu/Bu)	0/1 (alive)
Nijeboer et al. (2015)	I	2 of 61	surgery + CTx	no	NA	NA	2/2 (both died at 1-2m post-HCT)
Chonabayashi et al. (2007)	I	1 of 1	2 # EPOCH → PD → 4 # ICE	no	PR	RIC (Flu/Mel/TBI 4Gy), double cord blood	0/1 (alive at 11m post-HCT)
Malamut et al. (2013)	I	3 of 37	surgery + CTx	no	NA	NA	1/3 (1 relapsed and died at 9m; 2 alive at 38m and >13m post-HCT)
Pt. reported here	II	1 of 1	6 # CHO(E)P + ASCT 1. relapse → 2 # DHAP + 4 # DHAox 2. relapse → 1 # pralatrexate PD → 2 # mini-BEAM	yes	CR	RIC (Flu/Bu/ATG)	suspected relapse at 6m post-HCT (alive)

Abbreviations: EATL = enteropathy-associated T-cell lymphoma; MEITL = monomorphic epitheliotropic intestinal T-cell lymphoma; # = cycle; CHO(E)P = cyclophosphamide, adriamycin, vincristine, (etoposide), prednisolone; EPOCH = etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; ICE = ifosfamide, etoposide, carboplatin; DHAP = dexamethasone, cytarabine, cisplatin; DHAox = dexamethasone, high-dose cytarabine, oxaliplatin; PD = progressive disease; mini-BEAM = carmustine, etoposide, cytarabine, melphalan; RIC = reduced intensity conditioning; Flu = fludarabine; Cy = cyclophosphamide; Bu = busulfan; Mel = melphalan; TBI = total body irradiation; ATG = anti-thymocyte globulin; CTx = conventional chemotherapy; ASCT = high-dose CTx + autologous HCT; CR = complete remission; PR = partial remission; NA = not available

were achieved. Immunosuppression was tapered and discontinued on d+55 (MMF) and d+193 (CsA), respectively. 18F-FDG PET-CT scan at 3m post-HCT showed CR, but at 6m relapse was suspected (under work-up). Only few cases of patients with EATL/MEITL treated with allo-HCT are reported in the literature (n = 9, Table 1), and the value of this highly aggressive therapy is not clear at this point. Of note, the patients listed in Table 1 were given allo-HCT instead of ASCT. Long-term complete remission (CR) could be achieved in 4/9 patients, while 5 patients suffered from early relapse and died of the disease (n = 4 before d+100 post allo-HCT). ASCT following surgery and CTx appears to cure 33–60% of patients in available series. No treatment concept is available for relapse following ASCT, and no published data are available for allo-HCT for relapse post ASCT. The disease is exceedingly rare and is afflicted with very poor outcomes. Therefore, patients given this aggressive treatment should be reported, even when treatment outcomes are not positive.

**Disclosure of conflict of interest:** None.

**P625**  
**Strong graft versus lymphoma effects with low toxicity of haploidentical hematopoietic stem cell transplantation comparing with HLA-identical in T cell lymphomas: A retrospective multicenter study**

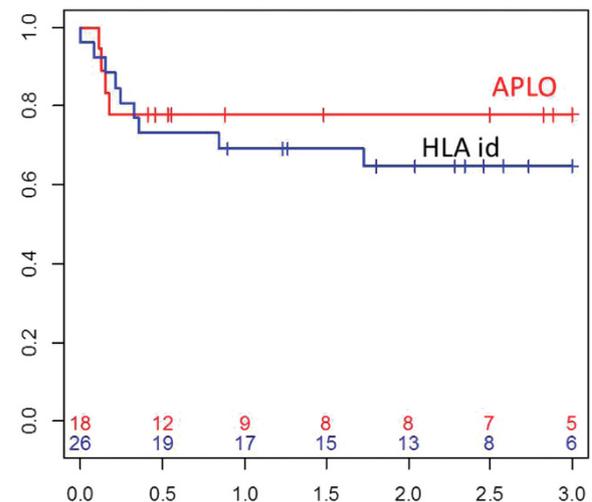
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Consolidation treatment of relapsed/ refractory T-NHL with allogeneic stem cell transplant (SCT) is considered a curative options but few patients manage to undergo this procedure, due to the highly refractory nature of the disease. The primary aim of this work is to evaluate the GVL effects among T-NHL with both HLA identical and Haploidentical donors. We have retrospectively analyzed the long term outcome of 43 consecutive patients affected by T-NHL, received HLA-identical or T-cell replete haplo-SCT with PT-Cy, in 2 european centers, between February 2010 and October 2015. The patients received nonmyeloablative (NMAC) or Reduced intensity (RIC) conditioning regimen. GVHD prophylaxis consisted of 50 mg/kg of PT-Cy (day +3 and +4) in haplo setting and ATG plus cyclosporine A in the HLA identical setting.

Patients characteristics were reported in the Table 1. No differences were founded in the two groups . Most of the patients were transplanted in complete remissions but only 8 as consolidation of first line. No graft failure occurred. The cumulative incidence of acute GVHD grade 3–4 was 5% in the haplo setting vs 11% in the Hla id .Extensive chronic GVHD was seen in 7% of haplo, and in 28% in the HLA id . 9 patients had CMV reactivation, 3 hemorrhagic cystitis, and 1 EBV reactivation. After a median follow-up of 3 years OS was 83% and 71% and PFS was 77% and 64% in the haplo vs Hla id group see Figure 1. NRM was 5% in Haplo setting and 11% in HLA identical one. The 3 years CIR is 16% and 23% in Haplo and Hla id setting respectively. This study confirm a strong anti-lymphoma effect of allo HSCT without prohibitive toxicities. Haplo-HSCT with PT-Cy shows low rate of cGVHD in a contest of poor prognosis T-NHL patients.

[P625]

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**Disclosure of conflict of interest:** None.

Table1. Patients Characteristics

All	Haplo	HLAid	
<b>N</b>	43	18	24
<b>Sex</b>			
<b>M</b>	32(77%)	14(78%)	18(75%)
<b>F</b>	10(23%)	4(22%)	6(15%)
<b>Stage at diagnosis</b>			
<b>Stade I-II</b>	12(31%)	4(22%)	8(33%)
<b>Stade III-IV</b>	30(69%)	14(78%)	16(67%)
<b>Previous HDC</b>	12 (28%)	5 (28%)	7 (29%)
<b>In first remission</b>	8(18%)	3(10%)	5(20%)
<b>At relapse</b>	4(9%)	2(11%)	2(8%)
<b>Histology</b>			
<b>T NOS</b>			
<b>TLAlic</b>	13(28%)	7(33%)	6(29%)
<b>Mycosis</b>	13(30%)	5(27%)	9(11%)
<b>Fungoides</b>	3(7%)	1(5%)	2(8%)
<b>Extranodal N/K</b>	1(2%)	0	0
<b>ALK +</b>	7(16%)	2(11%)	5(21%)
<b>ALK-</b>	4(9%)	3(16%)	1(4%)
<b>Panniculitis</b>	1(2%)	0	1(4%)
<b>Gamma/delta</b>	1(2%)	1(5%)	0
<b>Disease status at allo</b>			
<b>CR</b>	33(76%)	21(84%)	12(50%)
<b>CR1</b>	12(27%)	7(28%)	5(20%)
<b>CR2</b>	21(49%)	14(56%)	7(29%)
<b>PR</b>	6(14%)	3(12%)	3(12%)
<b>PD/SD</b>	3(7%)	1(4%)	2(8%)
<b>Median age at transplant</b>	55(21-68)	59(20-68)	48(21-68)
<b>Indication for allo</b>		4(22%)	4(17%)
<b>First line</b>	8(19%)		
<b>Tandem auto allo</b>		1(6%)	1(4%)
<b>Relapse after auto</b>	2(5%)	4(22%)	7(29%)
<b>Plurirefractory (never in CR)</b>	11(26%)	2(11%)	3(7%)
<b>Plurirelapsed</b>	5(11%)	7(39%)	9(21%)
<b>HCT-CI</b>			
<b>0-1</b>	8 (19%)	5(28%)	4(17%)
<b>2</b>	9(21%)	2(11%)	6(25%)
<b>&gt;3</b>	25(59%)	11(61%)	14(58%)
<b>Stem cell source</b>			
<b>BM</b>	7 (16%)	5(28%)	2(8%)
<b>PBSC</b>	37 (84%)	13 (72%)	24 (92%)
<b>DRI</b>			
<b>Intermediate</b>	37(84%)	12(66%)	25(96%)
<b>High</b>	7(16%)	6(33%)	1(4%)

**P626****Successful bridge with Ibrutinib monotherapy to allogeneic stem cell transplant in relapsed mantle-cell lymphoma**

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Ibrutinib is the first-in class Bruton Tyrosine Kinase inhibitor that has been approved for the treatment of relapsed mantle cell lymphoma. However, despite the high response rate of 68% including 21% of complete response, the median duration of response is relatively short with an overall survival of 58% at 18 months (Wang ML, et al, N Engl J. Med 2013). We report a single experience of 3 patients with relapsed MCL who underwent allogeneic stem cell transplant (AlloSCT) after Ibrutinib monotherapy salvage. All 3 patients had previous autologous stem-cell transplantation (ASCT) before and were given Ibrutinib at a dose of 560 mg daily after the second or subsequent relapse. All patients had to be at least in PR according to Cheson 2014 criteria before AlloSCT. Patients had an unrelated 10/10 (2) or 9/10 (1) Allo-SCT from peripheral hematopoietic stem cells after a reduced conditioning regimen with Busilvex, Fludarabine and antithymocyte globulin in association with Zevalin according to our recent published phase 2 study protocol (Bouabdallah K, et al. Ann Oncol 2015). Graft versus host disease (GVHD) prophylaxis consisted on Ciclosporine and Methotrexate. Patients (2M/1F) were aged from 62 to 65 years and received between 2 and 3 previous chemotherapy regimens including ASCT in their last treatment strategy before introduction of Ibrutinib. All patients had extensive disease with gastric involvement in 2 patients and pulmonary localization in 1 patient. Median time between diagnosis and Ibrutinib introduction was 5 years (3–7) and the median time between ASCT and AlloSCT was 4 years (4–5). Median duration of Ibrutinib treatment was 5 months (5–6) and it was stopped one week before proceeding to Allo-SCT. It was not planned to restart BTK inhibitor after transplant. Patients were assessed for response after at least 3 months of treatment with Ibrutinib. At time of evaluation, all patients were in complete (2) or very good partial response (1) before AlloSCT. The patient in partial response had 92% tumor reduction with persistent gastric ulcer where histology examination shows CD5+ but negative cyclin D1 lymphoid cells. All patients engrafted (median duration of PNN <500G/L = 11 days (10–15) and median duration of platelets <20G/L = 2 days (0–14)) with full-donor chimerism at 1 month. One patient had a grade II cutaneous chronic GVHD (cGVHD) with favorable outcome and developed 8 months later a bronchopulmonary obstruction syndrome related to cGVHD. With a median follow-up of 9 months (9–23) after Allo-SCT, all 3 patients are alive in CR. One patient, in complete metabolic response before transplant had a gastric relapse 3 months later but achieved again a CR 3 months after reintroduction of Ibrutinib. After the first case reported by Furtado M et al (Leuk Lymphoma 2016), we report here 3 additional cases with longer follow-up after allogeneic transplantation. The excellent tumor control after treatment with Ibrutinib together with a very good outcome after AlloSCT should drive to consider this approach in young patients with MCL relapse after ASCT.

**Disclosure of conflict of interest:** None.

**P627****TEAM conditioning (Thiotepa, etoposide, cytarabine, melphalan) prior to autologous hematopoietic stem cell transplantation for Hodgkin and non-Hodgkin lymphoma: First results from a prospective multicenter study**

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Autologous hematopoietic stem cell transplantation (autoSCT) is considered the standard approach for high risk or relapsed/refractory non-Hodgkin and Hodgkin lymphoma. Although a large variety of conditioning regimens are available, including the widely used BEAM (carmustine, etoposide, cytarabine, melphalan), there is no consensus regarding a standard approach. In the context of carmustine shortage, we have chosen to replace it by thiotepa. However, clinical data about thiotepa-based autoSCT conditioning are still sparse, except some retrospective data for primary central nervous system lymphoma. Thus, we designed a multicenter prospective study (NCT02504190) to assess the efficacy and toxicity of a TEAM (thiotepa, etoposide, cytarabine, melphalan) conditioning regimen. TEAM regimen consisted in total dose thiotepa of 8 mg/kg on day-6; etoposide 100 mg/m<sup>2</sup>/12 h and cytarabine 200 mg/m<sup>2</sup>/12 h (day-5 to -2); melphalan 200 mg/m<sup>2</sup> on day-1. Patients underwent autoSCT with TEAM conditioning, and were included in this analysis if they have fulfilled the following criteria: age older than 18 years, biopsy-proven Hodgkin or non-Hodgkin lymphoma, HIV seronegative, and first autoSCT. Thirty-three male and nine female with a median age of 59 years (range: 19–72) were analyzed thus far. Karnofsky score was 20G/L was 13 days (range: 8–48). Of note, 5 patients received thrombopoietic agents after engraftment because of persisting thrombocytopenia. The most significant regimen-related toxicities were mucositis in 100% of patients (median grade = 3, range: 2–4) and diarrhea in 98% of patients (median grade = 1, range: 0–3). Other non-hematologic grade 3 adverse events occurred in 13 patients (31%) and no grade 4 adverse events were observed. Central line-associated bloodstream infection occurred in 11 patients (26%). Surprisingly, 16/36 evaluable patients (44%) developed human herpesvirus 6 reactivation. Only 2 patients required intensive care unit transfer. The median duration of hospital stay was 29 days (range: 20–62). After a median follow-up of 8 months (range: 2–20), the non-relapse mortality (NRM) was 0%. Only one patient relapsed of refractory AITL 2 months after autoSCT and died 1 month after. The estimated 1-year overall survival and progression-free survival were 98% and 98%, respectively. A TEAM conditioning regimen seems to be a safe and valid platform in autoSCT for patients with high-risk or relapsed/refractory lymphoma. Although mucositis and diarrhea were frequent, there were no grade 4 adverse events and no deaths related to the treatment. Updated results with updated follow-up will be presented.

**Disclosure of conflict of interest:** None.

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## P628

### The cell of origin has no prognostic impact on high-dose chemotherapy with R-beam and autologous stem cell transplant for diffuse large B cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is a biologically heterogeneous disease that can be classified according to its cell-of-origin (COO). The germinal center B-cell (GCB) subtype has better outcome with frontline R-CHOP than the activated B cell (ABC) subtype. However, the prognosis of these two types of DLBCL after high-dose chemotherapy and autologous stem cell transplant (ASCT) is less clear. The purpose of our study was to evaluate progression-free survival (PFS), event-free survival (EFS) and overall survival (OS) in a cohort of DLBCL patients treated with R-BEAM (Rituximab, carmustine, etoposide, cytarabine, melphalan) and ASCT according to COO. We have

retrospectively reviewed 72 patients with relapsed or primary refractory de novo DLBCL who received R-BEAM and ASCT from 2005 to 2015 at the MD Anderson Cancer Center. The COO was determined by immunochemistry using the Hans algorithm. Transformed, primary mediastinal and double hit/double expressor lymphomas were excluded. Our study was approved by the institutional review board. Median age at transplant was 63 years (28–74). 75% and 25% of patients were males and females, respectively ( $P=0.03$ ). Fifty-nine (82%) and 13 (18%) patients had relapsed and primary refractory chemosensitive DLBCL, respectively. Secondary IPI was 0–1 in 23 (44%) patients, 2 in 13 (25%) patients and 3–4 in 16 (31%) patients. Fifty-one (71%) and 21 (29%) patients had GCB and ABC tumors, respectively. ABC patients received more prior lines of chemotherapy than GCB patients (76% vs 48% received > 2 lines of chemotherapy,  $P=0.03$ ). The rest of characteristics were equally distributed between both groups (Table 1). Median follow up was 60 months (5–120). A total of 38 patients died: 12 (57%) patients in the ABC group and 26 (50%) in the GCB group. There were no differences in OS between GCB and ABC lymphomas (median OS 57 vs 78 months, HR: 1.2, 95% CI: 0.5–2.5). A total of 34 patients relapsed: 8 (38%) in the non-GCB and 26 (50%) in the GCB group. We found no differences in PFS (HR: 0.6, 95% CI: 0.3–1.3) or EFS (median EFS 31 vs 33 months, HR: 0.9, 95% CI: 0.5–1.7). Of note, the median time to relapse was 5 months in the GCB group compared to 17 months in the non-GCB. Disease status at transplant, PET results prior to transplant, secondary IPI or prior number of chemotherapy lines had no prognostic impact on OS. However, achieving a pre-ASCT metabolic CR was predictive of a more favorable EFS (HR: 2.3, 95% CI: 1.2–4.5,  $P=0.01$ ). Patients over 70 years had significantly worse OS (HR: 4.3, 95% CI: 1.9–9.8,  $P<0.001$ ) compared to younger patients. However, age did not affect PFS or EFS. Main causes of death in this group of patients were relapse (57%) and infection (29%). 11 (15%) patients developed secondary malignancies post autologous stem cell transplant; 8 patients were diagnosed with a therapy-related myelodysplastic syndrome, one patient with therapy-related AML, one with brain stem glioma and one with colon cancer. In this cohort of patients the COO failed to predict transplant outcomes, despite more prior chemotherapy lines in the ABC subgroup.

[P628]

	ABC (n=23)		GCB (n=49)		P-value
Age (median)	63 yrs	(28-72)	63 yrs	(40-74)	
23-35	6	26%	13	26%	0.8
36-45	8	35%	14	27%	
46-55	4	17%	15	31%	
56-74	5	22%	11	22%	
Gender					0.08
Male	11	48%	15	31%	
Female	10	43%	34	70%	
History					0.3
Disordered	2	9%	15	31%	
None	19	81%	34	69%	
Response at SCT					0.1
Yes	2	9%	18	37%	
No	19	81%	31	63%	
Unknown	0	0%	2	4%	
Prior chemotherapy					0.08
<2	8	35%	28	57%	
>2	15	65%	21	43%	
Secondary IPI					0.8
0-1	7	30%	16	33%	
2	4	17%	9	18%	
>2	9	39%	11	23%	
Prior Radiotherapy					0.4
Yes	4	17%	14	29%	
No	17	73%	35	71%	
Overall-free interval					0.8
< 12 months	11	48%	20	41%	
>12 months	10	43%	31	63%	
Indication for SCT					0.1
Rel <12m	4	17%	6	12%	
Rel >12m	4	17%	21	43%	
Rel >12m	11	48%	19	39%	

**P629****The DICEP regimen effectively reverses the poor outcome for lymphoma patients with suboptimal response or failure post 1st salvage treatment**

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The outcome of patients (pts) with refractory Hodgkin's (HL) and non-Hodgkin lymphomas (NHL) post 1st salvage treatment (salv1) is considered poor. The published data, have shown extremely low survival rates (15–20%) even after 2nd salvage treatment (salv2) followed by autologous stem cell transplantation (ASCT), due to the low response rates post salv2 and the high relapse rates post ASCT, confirming that the management of these pts remains a major challenge. We herein evaluated the DICEP regimen [Dose Intesified Cyclophosphamide (1750 gr/m<sup>2</sup>), Etoposide (350 mg/m<sup>2</sup>) and Cisplatin (35 mg/m<sup>2</sup>), days 1–3] as a salv2 treatment, in terms of safety and efficacy regarding disease response and stem cell mobilization/collection. Moreover, we evaluated pts' long term outcome post ASCT. We retrospectively analyzed the data of 27 (11 HL, 16 NHL) pts, with a median age of 32 (16–61) yrs. Twenty-one had suboptimal response (75% reduction): 4 and minor response (≤50% reduction): 2). Three pts had stable disease while 3 experienced progression. Overall 23/27 pts underwent ASCT after a median of 44 days (range: 22–70) post DICEP. No pt was considered ineligible for the ASCT due to unacceptable toxicity post DICEP; 4 did not undergo ASCT because of progressive (*n* = 3) or stable (*n* = 1) disease. The 5-yr overall survival (OS) was 70% for the whole cohort of pts (71% for HL and 66% for NHL, *P* = ns) while the 4-yr progression free survival (PFS) from DICEP administration (± ASCT) was 62% (60% for HL and 64% for NHL *P* = ns). In particular, for the 23 autografted pts, the 5-yr OS was 70% (80% for HL, 70% NHL *P* = ns) and the 4-yr PFS was similar, 70% (65% for HL, 72% for NHL, *P* = ns) Our data demonstrate that DICEP is an effective salvage regimen with acceptable toxicity and no negative impact on the CD34+ collection. The promising response rates post DICEP in combination with the very encouraging PFS rates achieved post ASCT, in this unfavorable and heavily pretreated group of patients, strongly support the rationale for using DICEP as 1st line salvage regimen in selected pts in order to proceed to a successful ASCT.

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**P630****Treosulfan-based conditioning and sirolimus for prevention of GvHD after allogeneic transplantation in advanced lymphomas: Results in 73 patients**

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Advanced lymphomas still represent a therapeutic challenge and allo-HSCT is among treatment options. Between March 2007 and August 2016, seventy-three patients (pts) affected by R/R Lymphomas (34 NHL and 39 HL) underwent an allo-HSCT after a Treosulfan-based conditioning regimen and Sirolimus as calcineurin-inhibitor-free prophylaxis of GvHD. Six pts received a MRD, 18 pts a MUD, and 49 pts a HAPLO

unmanipulated PBSC. At allo-SCT 30 pts were in CR, 13 pts were in PR, and 30 pts had SD/PD; sixty patients underwent autologous SCT before allo-HSCT. HCT-CI was evaluable for 64 pts, 33 had a score ≥3. Thirty-three pts received Treosulfan and Fludarabine reduced toxicity conditioning regimen (RTC) and intensification with other alkylating agent or with 4 Gy Total Body Irradiation was added on the remaining 40 pts (Myeloablative Conditioning, MAC). All pts received a backbone GvHD prophylaxis with Sirolimus and Mycophenolate Mofetil; ATG or PT-Cy or both were added in 41, 25, and 3 pts respectively. Median numbers of infused CD34+/Kg and CD3+/Kg were 6.01 × 10<sup>6</sup> (range: 2.72–9.06) and 2.39 × 10<sup>8</sup> (range: 0.3–6.89), respectively. Median follow-up was 44 months (range: 3–111); median time to neutrophil ≥ 0.5 × 10<sup>9</sup>/L was 17 days, and 20 days to platelet ≥ 20 × 10<sup>9</sup>/L. Sixteen out of 43 patients with pre-transplant active disease obtained a CR after Treosulfan conditioning; nine of them (6 HL and 3 B-NHL) achieved durable CR without post transplant treatment. One- and 3-years OS was 62% and 48%, PFS was 47% and 37% at 1 and 3 years respectively; cumulative incidence of relapse/progression was 32% and 42% at 1 and 3 years. GRFS was 31% and 19% at 1 and 3 years, respectively. Transplant Related Mortality (TRM) was 15% at 100 days, 21% at 1 year and for the entire follow-up. The 100-day Cumulative Incidence (CI) of aGvHD grade ≥ 2 was 19% and CI of aGvHD grade ≥ 3 was 10%; CI of moderate to severe cGvHD was 23% at 2 years and for the entire follow-up. No differences in CI of aGvHD or cGvHD were found if pts were stratified according to donor type, but CI of moderate-severe cGvHD was significantly higher in pts after MAC regimens (*P* < 0.0005). As expected, the outcome of pts in CR was significantly better compared with active disease, in terms of OS (*P* = 0.0061), PFS (*P* = 0.00022), RI (*P* = 0.0028). In multivariate analysis, intensity of conditioning regimen (RTC vs MAC), GvHD prophylaxis (use of ATG, PT-Cy or none), donor sex and age at allo-SCT did not impact the transplant outcomes; both OS and PFS were reduced by active disease at allo-HSCT (HR = 4.37, CI 95% 1.76–10.86, *P* = 0.01 and HR = 4.37, CI 95% 1.97–9.7, *P* = 0.00, respectively) and by NHL histology (HR = 3.88, CI 95% 1.65–9.19, *P* = 0.02 and HR = 2.43, CI 95% 1.09–5.42, *P* = 0.03, respectively); GRFS and RI were impacted only by active disease (HR = 2.25, CI95% 1.19–4.25 and HR = 4.89, CI 95% 1.87–12.62, *P* = 0.01, respectively). Allo-HSCT after Treosulfan conditioning and Sirolimus GvHD prophylaxis is feasible even in heavily pretreated pts affected by lymphomas. Complete remission status before transplant remains crucial for better outcomes and in the era of new targeted treatments should be pursued.

**Disclosure of conflict of interest:** None.

**P631****Upfront autologous stem cell transplantation in patients with diffuse large B cell lymphoma: Focused on risk factors for survival and conditioning regimens**

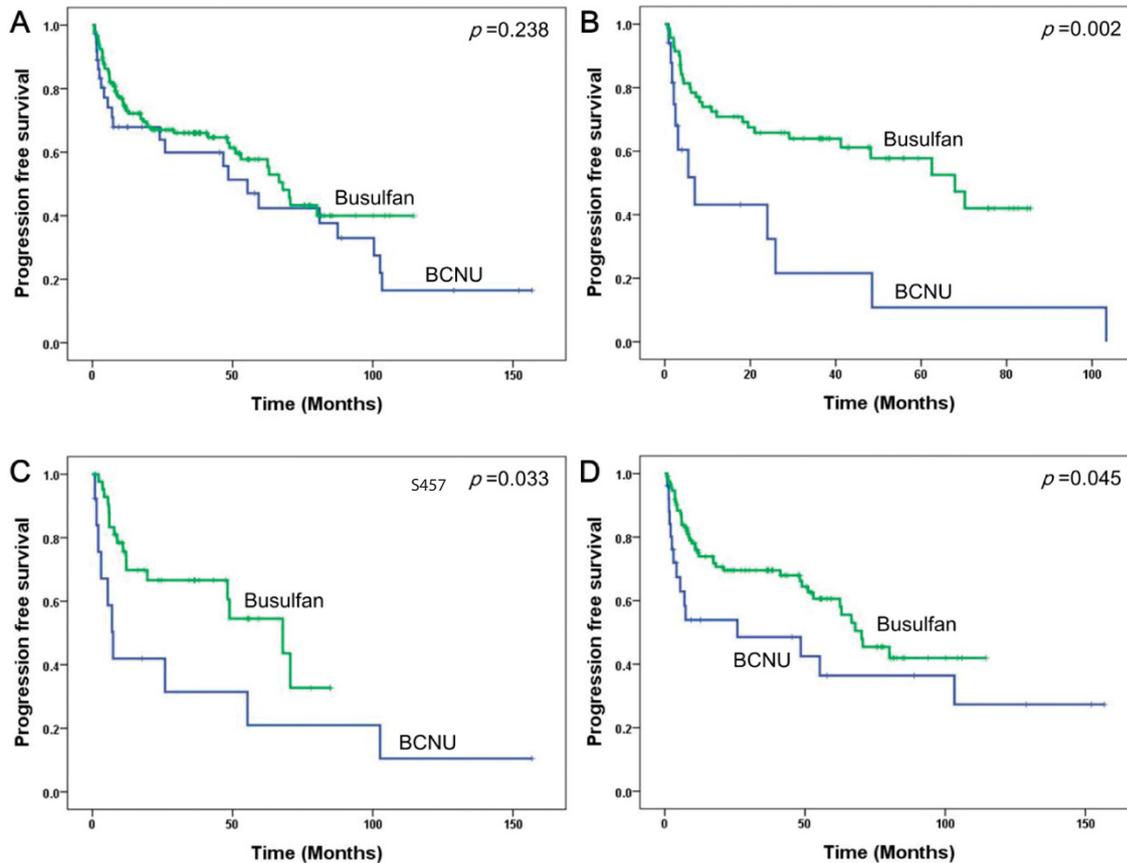
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For the treatment of aggressive lymphoma, high dose chemotherapy followed by autologous stem cell transplant (ASCT) is an important component. However, the role of upfront ASCT in patients with diffuse large B cell lymphoma (DLBCL) is still controversial. Furthermore, there is currently no consensus on a single best conditioning regimen for ASCT in patients with DLBCL. We retrospectively analyzed the records of 184 patients with DLBCL who underwent upfront ASCT in state of complete remission (CR) or partial remission (PR) from 17 institutions in Korea. We evaluated the outcomes and prognostic factors of upfront ASCT in patients with DLBCL. We compared the outcomes of most widely used two conditioning regimens for ASCT; carmustine based regimens and busulfan containing regimens. Total 141 patients (81.0%)

achieved CR after ASCT and overall response rate (ORR) was 86.7%. With median follow up of 34 months, 65 patients (35.3%) had progression or relapse. The 3-year overall survival (OS) rates and progression free survival (PFS) rates were 75% and 65%, respectively. Infection events were found in 99 patients (54.5%) and treatment related mortality was 3.8%. These outcomes were comparable with the results of previous other studies. Cox multivariate analysis for OS showed that Eastern Cooperative Oncology Group performance status (ECOG PS)  $\geq 2$  ( $P=0.003$ ) and rituximab based induction therapy ( $P=0.062$ ) were significant prognostic factors. In addition, the following factors were significantly associated with PFS in multivariate analysis; female ( $P=0.031$ ), PS  $\geq 2$  ( $P=0.018$ ) elevated  $\beta 2$ -microglobulin ( $P=0.008$ ), failure to achieve CR with induction chemotherapy ( $P=0.004$ ), carmustine based conditioning regimen ( $P=0.012$ ) and melphalan based conditioning regimen ( $P=0.031$ ). There were no significant differences in OS and PFS according to stage, B symptom, bulky disease, high lactate dehydrogenase, bone marrow involvement, high or high-intermediate international prognostic index (IPI), absolute lymphocyte count and absolute monocyte count. Therefore, it is considered that upfront ASCT can overcome the poor prognosis in patients with advanced stage or high risk IPI. In the analysis with conditioning regimen, neutrophil and platelet engraftment were slower in the carmustine group compared to the busulfan group. There were no significant differences in OS between busulfan group and carmustine groups with 3-year OS rates of 74.4% and 77.9%, respectively ( $P=0.797$ ). PFS at 3-years was 66.0% in busulfan group versus 59.9% in carmustine group ( $P=0.241$ ). However, carmustine based conditioning regimen was poor prognostic factors for PFS in multivariate



analysis ( $P=0.004$ ). In subgroup analysis, busulfan group had significantly higher PFS compared to the carmustine group especially in female patients (67.9 months vs. 7 months,  $P=0.002$ ), with B symptom (67.9 months vs. 7.4 months,  $P=0.033$ ) and abnormal serum LDH level (70.2 months vs. 25.9 months,  $P=0.045$ ). The outcomes of upfront ASCT in patients with DLBCL after induction therapy were acceptable. It is considerable in selected high risk patients who achieve CR with induction treatment, and have good performance status at diagnosis. In cases of conditioning regimen, busulfan based regimen resulted in improved outcomes compared with carmustine based regimen especially in patients with disseminated disease or female patients.

**Disclosure of conflict of interest:** None.

## Multiple myeloma

### P632

#### Association between grade of response and outcomes in transplant-eligible myeloma patients

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Outcome of patients with multiple myeloma (MM) has improved; however, subsets of patients do worse than expected. In transplant-eligible patients, achievement of complete response (CR) versus. From 2004 to 2014 (median follow-up of 4.17 years), 140 patients with a median age of 60 years (range: 37–69), received ASCT as first line treatment for MM. Most of them were male (74 men, 66 women) and 22.9%, 35%, 42.1% scored I,II and III in the ISS, respectively. The most

frequent heavy chain was IgG (47.9% IgG, 24.3% IgA, 2.1% IgD; no heavy chain was present in 25%). The predominant light chain was kappa (60%). 80 patients had Bence-Jones positive myeloma. 109 received bortezomib as induction therapy before transplant. We analyzed overall survival (OS) and progression-free survival (PFS) in 5 groups of patients. We separated the groups according to improvement in grade of response from preASCT to postASCT. The post-ASCT grade of response was measured 3 months after ASCT. The OS and PFS were estimated by the Kaplan-Meier method. PFS was measured from diagnosis to disease relapse and OS was measured from diagnosis to death by any cause. Results by subgroups of patients are detailed in Table 1. Median OS and PFS of the whole group was 8 years and 54 months, respectively. If we analyze groups only by their grade of response before ASCT we find the following results: RC (5-years OS rate 78.9%, median PFS 95 months); PR/VGPR (median OS 7.31 years and PFS 40 months); SD/progression (median OS 6.46 years and PFS 21 months). According grade of response after ASCT, instead: RC (5 years OS rate 78%, median PFS: 66 months); PR/VGPR (median OS 7.31 years, and PFS 40 months); SD/Progression (median OS 1.05 years and PFS 11 months). In our experience, the grade of response before ASCT is a capital predicting factor for patients OS and PFS. Patient in CR before ASCT that preserve it after transplant, have a median PFS of 95 months, the 5 years OS rate being 80.4%. Patients in situation of progression after ASCT have a very dismal prognosis (median OS 1.05 years, PFS: 11 months), however, patients who change from SD/progression to PR after ASCT have a median PFS of 29 months and a OS of 6.4 years. Comparing these results we observe that this second group is particularly benefited by transplant.

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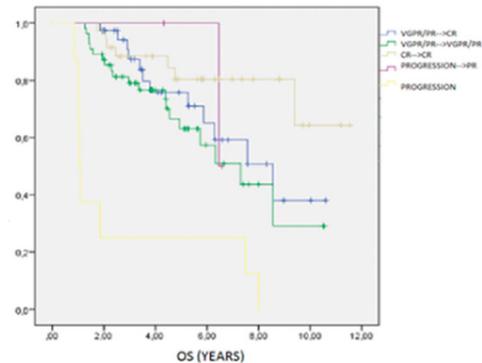
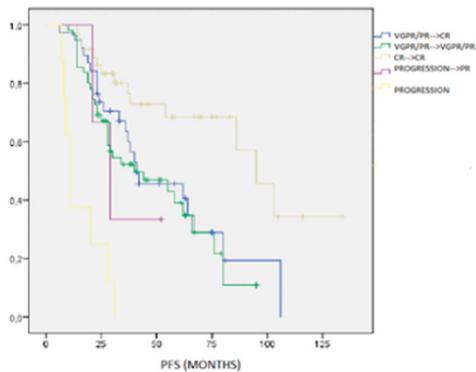
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**Disclosure of conflict of interest:** None.

[P632]

	CR→CR(36)	VGPR/PR→>CR(38)	VGPR/PR→>VGPR/PR(55)	SD/PROGRESSION→PR(3)	PROGRESSION (8)
<b>GENDER:</b>	M(26) F(11)	M(17) F(21)	M(28) F(27)	M(0) F(3)	M(4) F(4)
<b>HEAVY CHAIN</b>					
IgG	7	14	41	3	2
Others	29	24	14	0	6
<b>LIGH CHAIN</b>					
Kappa	18	22	35	2	7
Lambda	16	14	20	1	1
Negative	2	2	0	0	0
<b>BENCE JONES</b>					
Positive	22	22	35	0	7
<b>ISS</b>	I(11) II(7) III(18)	I(9) II(16) III(13)	I(11) II(22) III(22)	I(0) II(2) III(29)	I(1) II(3) III(4)
<b>PFS (months)</b>	95	41	40	29	11
<b>OS (years)</b>	*	8,5	7,3	6,4	1,0

CR: Complete response; PR: Partial response; VGPR: very good partial response; SD: stable disease  
M:Male; F:Female  
\* OS not yet reached. 5 years OS rate: 80.4%



**P633****Autologous peripheral blood hematopoietic stem cell transplantation in elderly patients with multiple myeloma as a standard therapeutic procedure. Is it feasible? A single-center experience**

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Autologous peripheral blood stem cell transplantation (PBSCT) represents a standard therapeutic approach in the treatment protocol of myeloma patients. It is known that multiple myeloma is a hematological disease that is a characteristic for the older population. Autologous PBSCT ideally should be performed in every myeloma patient, but with the elderly myeloma patients the procedure might be risky if know the possible comorbidities, or the possibility of the body to fully compensate the side effects of the conditioning regimen, the procedure or its possible complications. We present our experience in using high dose conditioning with Melphalan 200 mg/m<sup>2</sup> followed by autologous PBSCT for elderly myeloma patients, using the age limit of 65 years. Our retrospective analysis of our data during 16 years of experience, shows that we have performed autologous PBSCT on 12 patients with myeloma at the age of 65 or older. 11 males (91.6%), and 1 female (8.4%). 6 patients (50%), were diagnosed with IgG type myeloma, 5 patients (41%) with IgA myeloma, and 1 patient (9%) with light chain myeloma. Median age of the patients was 66.9 years (65–73). All patients were initially treated with Cy-Thal-Dex regimen. In 5 (41%) patients complete response (CR) was achieved, in 5 (41%) very good partial response (VGPR), and in 2 (18%), partial response (PR). In all patients the mobilisation of hematopoietic stem cells was performed with G-CSF, and a median of 2 apheresis procedures were performed, and the average number of collected cells was  $3.11 \times 10^8$ /kg TT mononuclear cells (range: 6.0–2.1). Days to confirmed engraftment in our group of patients was 11.5 (range: 9–15). The number of blood transfusions was on average 2.8 (range: 0–6), and the number of transfusion of thrombocytes 38.1 units (range: 10–74). In the majority of patients, mainly after the year 2012 (that represents 10 patients of the whole group), we used noncryopreserved hematopoietic stem cells, kept under the temperature of 40C, for median of 2 days, thus avoiding the toxicity of DMSO. Additionally, we used central venous catheter inserted in the femoral vein for apheresis and application of the stem cells afterwards. The day after, the catheter was removed, thus avoiding catheter associated infections. All patients received standard infectious prophylaxis with fluconazole 200 mg/daily, ciprofloxacin 500 mg/ two times daily, acyclovir 200mg/ three times daily, cefixime 400 mg/once daily, and ursodeoxycholic acid for VOD prevention. No serious infectious complications were reported. Our transplant related mortality was 0%. In the group with noncryopreserved stem cells no graft failure was reported. In two patients we even performed tandem autologous PBSCT with no major complications. Of the group of 12 patients, the majority, 8 patients (66%), had HTA as comorbidity, 1 (8%) with cardiomyopathy, and 1 (8%) with inserted prosthetic aortic valves. Three patients (24%) have died because of relapse of the disease. Our oldest patients were 72 and 73 years old, and are still alive 1 year posttransplant, in CR. We can conclude the performing autologous PBSCT in elderly myeloma patients can be safe and effective therapeutic option, but with careful selection of the patients, balancing the risk profile of the patient and the benefit, or the risk of the procedure. Affective supportive care, monitoring and reducing the risk of complications is an imperative to a good result.

**Disclosure of conflict of interest:** None.

**P634****Autologous stem cell transplantation program for patients with multiple myeloma in an outpatient setting**

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The first and second authors contributed equally.

High-dose chemotherapy with melphalan and autologous blood stem cell transplantation (ABST) for treatment of symptomatic multiple myeloma (MM) is performed in the USA and Canada mostly on an outpatient basis, whereas in Germany and Western Europe an inpatient setting is the standard. We report on a German single-centre program to offer the procedure on an outpatient basis to selected patients. Major inclusion and exclusion criteria for eligibility were defined as follows: patients had to be able to reach the hospital within 45 minutes, had reliable support from their family at home, had an ECOG performance score of 0–1 and were willing and able to comply with the demands of the program. Patients with severe co-morbidities were not included. All patients were treated on our outpatients' clinic and examined on daily visits by a team of physicians. Feedback from patients was obtained by means of a questionnaire. From September 2012 to September 2016, 26 patients with MM stage IIIA were enrolled. All engrafted within the expected time range: (median time to leukocyte > 1,000 /μl and neutrophil recovery > 500 μl/l: 14 days; median time to platelet recovery > 20/nl: 10 days, > 50 /nl: 14 days). Twenty patients (77%) had an episode of neutropenic fever but only in 5 patients (19%) blood cultures were found to be positive. There occurred no cases of infection with multiresistant bacteria. Although rather liberal criteria for hospital admission were applied, 18 of 26 patients (69%) could be treated entirely on an outpatient basis. Eight patients (31%) were temporarily admitted for inpatient treatment with a median duration of 4.5 days (range: 2–18 days), mainly because of neutropenic fever. No severe adverse events occurred. Feedback from patients revealed a high level of satisfaction with the outpatient setting. High-dose chemotherapy and ABST on an outpatient basis is safe and feasible if conducted in a comprehensive surveillance program. The feedback from patients was very positive, thus encouraging further continuation and expansion of the program.

**Disclosure of conflict of interest:** None.

**P635****Blood graft composition and post-transplant recovery in myeloma patients mobilized with plerixafor**

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Table 1. Graft cellular composition according to the use of plerixafor.

Variable	Collected grafts			Infused grafts		
	Patients mobilized w plerixafor (n = 10)	Patients mobilized w/a plerixafor (n = 77)	p value	Patients mobilized w plerixafor (n = 10)	Patients mobilized w/a plerixafor (n = 77)	p value
CD34 <sup>+</sup> w/a 7-AAD (x10 <sup>6</sup> /kg)	4.9 (1.9-13.1)	5.0 (1.7-17.4)	0.545	3.2 (1.9-7.6)	3.2 (1.0-10.3)	0.680
CD34 <sup>+</sup> w 7-AAD (x10 <sup>6</sup> /kg)	3.2 (1.2-10.0)	3.6 (0.2-14.3)	0.284	1.8 (1.2-4.7)	2.4 (0.2-7.2)	0.581
CD34 <sup>+</sup> CD133 <sup>+</sup> CD38 <sup>-</sup> (x10 <sup>6</sup> /kg)	0.1 (0.04-0.75)	0.09 (0.005-106.0)	0.754	0.08 (0.04-0.35)	0.07 (0.005-103.0)	0.269
Proportion of CD34 <sup>+</sup> CD133 <sup>+</sup> CD38 <sup>-</sup> from all CD34 <sup>+</sup> cells (%)	4.3 (2.6-7.5)	3.0 (0.3-22.1)	0.001	3.1 (1.9-5.6)	1.9 (0.2-11.1)	<0.001
CD3 <sup>+</sup> (x10 <sup>6</sup> /kg)	292.7 (58.3-683.6)	89.4 (5.5-496.5)	<0.001	210.6 (29.2-388.3)	54.8 (2.75-345.1)	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> (x10 <sup>6</sup> /kg)	206.2 (37.6-502.5)	54.8 (3.4-249.9)	<0.001	128.2 (18.8-290.3)	31.6 (2.1-156.4)	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> (x10 <sup>6</sup> /kg)	86.6 (21.2-194.1)	25.6 (1.5-242.9)	0.004	74.1 (10.6-194.1)	18.1 (0.75-195.0)	0.001
CD19 <sup>+</sup> (x10 <sup>6</sup> /kg)	17.6 (1.7-76.7)	2.2 (0.01-61.59)	0.001	14.1 (0.87-66.61)	1.2 (0.005-61.590)	<0.001
NK cells (x10 <sup>6</sup> /kg)	28.3 (2.3-65.3)	9.2 (0.5-144.7)	0.015	27.1 (1.15-59.86)	6.9 (0.24-144.7)	0.008

w/a = without; w = with, 7-AAD = 7-aminoactinomycin

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Autologous stem cell transplantation continues to have an important role in the treatment of patients with multiple myeloma (MM). In MM patients the most commonly used mobilization method is granulocyte-colony stimulating factor (G-CSF) ± cyclophosphamide (CY). Generally, up to 10–20% patients mobilize poorly with these methods and plerixafor may be used to enhance mobilization. The most important parameter of graft quality has usually been the number of CD34<sup>+</sup> cells, but there are also significant numbers of other cell subsets in the grafts and they may also be of special interest in regard to post-transplant recovery and outcome. For example, a higher number of lymphocytes and NK cells in the grafts has been associated with improved lymphocyte as well as NK cell recovery, respectively. The mobilization methods used seem to affect the graft composition. However, there is currently no prospective data on the effects of plerixafor on the graft composition, post-transplant hematological and immune recovery or outcome in patients with MM. Altogether eighty-seven patients with MM were included into this prospective study. Seventy-seven patients were mobilized with G-CSF ± CY (control group) and ten patients received also

plerixafor due to poor mobilization (plerixafor group). In the control group 57/77 (74%) and in the plerixafor group 3/10 (30%) of patients were mobilized with G-CSF+CY ( $P = 0.009$ ). There were no statistically significant differences between the groups according to age, gender, paraprotein type, initial ISS, induction therapy used or disease status at the time of mobilization. By IMWG risk stratification, there were more high risk patients in the plerixafor group (5/10 vs. 13/77,  $P = 0.066$ ). Cryopreserved graft samples were analyzed with flow cytometry for T and B cells (CD3/CD8/CD45/CD19) as well as for NK cells (CD3/CD16+CD56). Also, CD34<sup>+</sup> cell subclasses were analyzed (CD34/CD38/CD133). Complete blood counts were evaluated at +15 days, 1, 3, 6 and 12 months post-transplant. To evaluate immune reconstitution, flow cytometry of lymphocyte subsets (T, B, NK) was performed in a subset of patients at 1, 3 and 6 months after the graft infusion using the same antibody panel as for graft analysis. There were no significant differences between the groups in the number of CD34<sup>+</sup> cells in the grafts. Also, the median number of aphereses was two in the both groups ( $P = 0.086$ ). The proportion of the more primitive CD34<sup>+</sup> cells (CD34<sup>+</sup>CD133<sup>+</sup>CD38<sup>-</sup>) was significantly higher in the plerixafor group ( $P = 0.001$ ). In addition, the number of various lymphocyte subsets analysed was significantly higher in plerixafor group

(Table 1). There were no statistically significant differences in the course of hematological recovery. The recovery of blood CD3+CD4+ T cells was significantly faster in the plerixafor group at one at three months post-transplant. There was no significant difference in the progression-free survival (PFS) (log rank,  $P=0.408$ ) with the median follow-up time of 703 days in the plerixafor group and 882 days in the control group (0.099). In the present study plerixafor added to G-CSF ± CY seemed to significantly alter the cellular composition of autologous blood grafts in poorly mobilizing MM patients. Hematological recovery was comparable but the CD3+CD4+ T lymphocyte recovery was faster in the plerixafor group. The PFS was comparable between the groups.

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fees from Abbvie, Roche, Celgene, Amgen and Sanofi. The other authors declare no conflicts of interest.

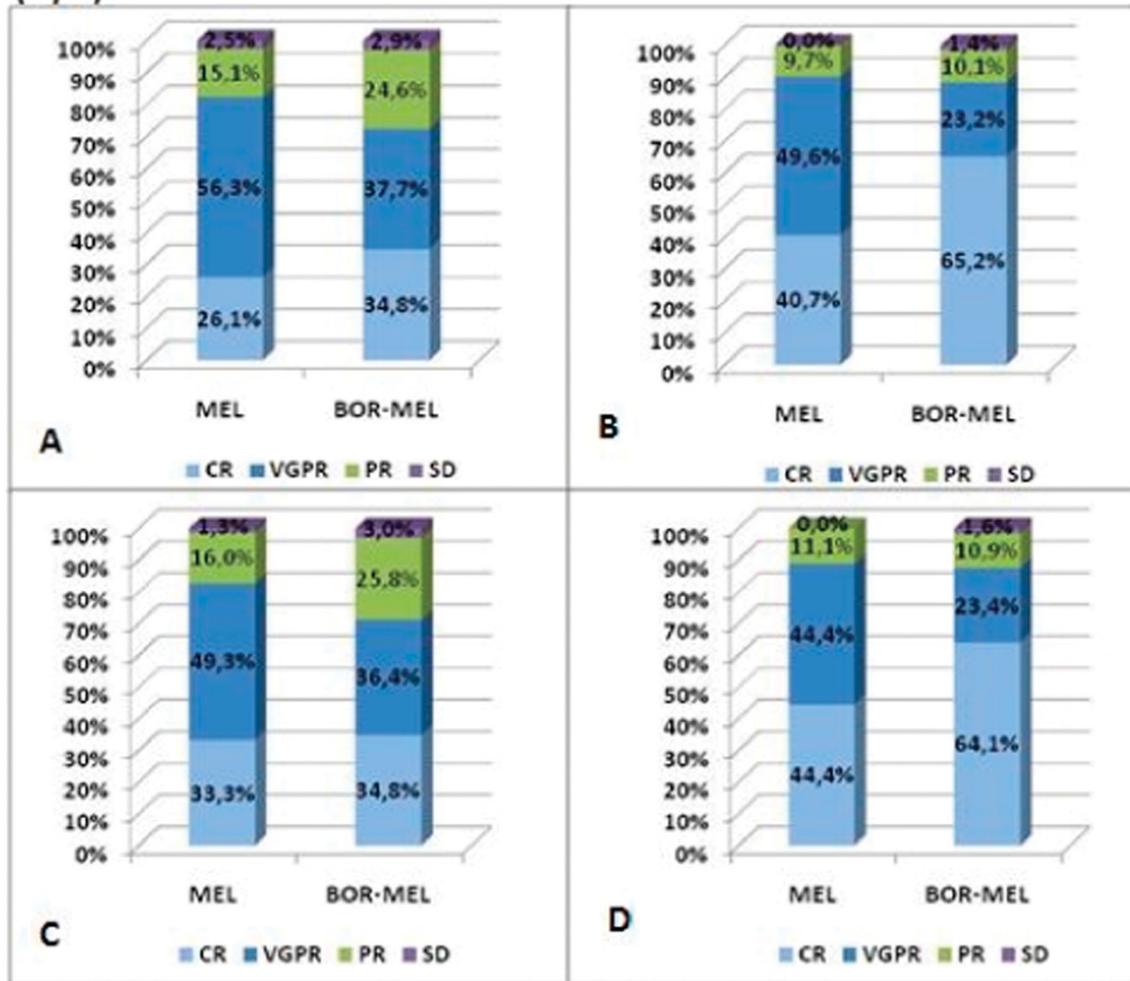
**P636**  
**Bortezomib after high-dose melphalan as conditioning regimen before autologous stem cell transplantation in patients with multiple myeloma: A comparison with the historical conditioning regimen with melphalan alone**

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High dose of melphalan followed by autologous stem cell transplantation (ASCT) is the standard of care for younger patients with Multiple Myeloma (MM). To enhance the efficacy of the conditioning regimen, the Intergroupe Francophone du Myelome added bortezomib to melphalan showing improved response rates, without significant toxicity. Bortezomib has shown synergistic effects with melphalan, mainly if the bortezomib is administered 24 hours after the melphalan. Since 2014, we have changed our conditioning regimen for patients with MM undergoing ASCT by adding bortezomib to

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**Figure 1. Response rate to induction therapy and pos-transplant for all patients (A y B) and for patients underwent bortezomib-based induction only (C y D).**



high dose of melphalan (BOR-MEL). We retrospectively analyzed 69 patients with MM who underwent ASCT between January 2014 and March 2016. In these patients, conditioning regimen consisted of a high dose of melphalan (140–200 mg/m<sup>2</sup>) intravenously on day -2 and two doses of intravenous bortezomib at 1.3 mg/m<sup>2</sup> administered on days -1 and +2. This cohort was compared with patients underwent ASCT between 2003 and 2013, conditioned with high dose of melphalan alone. Response rate was evaluated according to IMWG criteria. All patients were evaluated after induction therapy and 3 months after ASCT. All patients were followed until death or reference date (November, 2016). Results: Patients' demographics and baseline disease-related characteristics are shown in Table 1.

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Variable	MEL n=119	BOR-MEL n=69	P
Age, median, years(range)	57(33-72)	61(32-74)	0.016
Sex, males, n(%)	73(61.3)	42(60.9)	0.947
ISS-III, n(%)	36(36.4)	17(31)	0.503
Bortezomib-based induction, n(%)	75(63)	66(95.7)	<0.001
CD34+dose, cells/kgx10 <sup>6</sup> , median(range)	3.8(1.2-15.7)	3.1(1.75-12.6)	0.049
Neutrophil engraftment, median, days (range)	11(8-50)	11(9-18)	0.802
Platelet engraftment, median, days (range)	13(9-270)	14(10-71)	0.079

No difference was found in terms of neutrophil and platelet engraftment, hospitalization days (p=0.723) and use of mechanical invasive ventilation (p=0.415). BOR-MEL regimen did not enhance severity of preexisting peripheral neuropathy (PN) in any patients, and only one presented de novo grade 2 PN. Non relapsed mortality was 1.4% and 1% in the BOR-MEL and MEL cohorts, respectively (p=0.673). Complete response rate after transplant was significantly better in the BOR-MEL cohort than in the MEL cohort (65.2% vs. 40.7%; p=0.001) (Figure 1B). When the analysis was restricted to patients who received bortezomib-based therapy, this difference was also statistically significant (64.1% vs. 44.4%; p=0.024) (Figure 1D). Median of follow-up was 12 months in the BOR-MEL vs. 42 months in the MEL cohort. No difference was found in terms of overall survival (OS) and progression free survival (PFS) between both groups. For all patients, a post-transplant deeper response was associated with better OS and PFS (p=0.008 and p < 0.001, respectively). Our results are in line with previous studies demonstrating that bortezomib combined with melphalan is a well tolerated conditioning regimen and may enhance the response rate after transplant, even in patients receiving bortezomib in the induction therapy. These results should be confirmed in a randomized trial.

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**Disclosure of conflict of interest:** None.

#### P637

##### **Bortezomib-lenalidomide-dexamethasone versus bortezomib-cyclophosphamide-dexamethasone for newly diagnosed multiple myeloma patients eligible for autologous stem cell transplantation: A single center experience**

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For newly diagnosed patients (pts) with Multiple Myeloma (MM), the triple-agent induction treatment based on bortezomib plus dexamethasone in combination with cyclophosphamide (VCD) or lenalidomide (VRD) represent extremely reliable regimens, which in combination with early autologous stem cell transplantation (ASCT) result in high response rates and prolonged long-term outcomes. However, though both regimens are widely used, there are extremely limited studies that compare the VRD vs. VCD in terms of safety and efficacy. In the present study we compared the outcomes of 19 newly diagnosed MM pts who received induction treatment VRD (n=10) or VCD (n=9) and proceeded early to ASCT. The VRD and VCD pts groups were similar regarding age at diagnosis (52 vs.54 ys, P=ns), interval between diagnosis-ASCT (5,8 vs. 5,7 months, P=ns) and maintenance treatment post ASCT (8 vs. 6 pts, P=ns). Per Revised International Scoring System (RISS), the VRD-group had slightly more advanced disease (stage I: 1, stage II: 7 and stage III: 2), compared to VCD-group (stage I: 3, stage II: 5 and stage III: 1), however this difference was not statistical significant. The conditioning regimen consisted of single agent melphalan: 200mg/m<sup>2</sup>. The t-test, Kaplan– Meir and Cox regression were utilized for the statistical analysis. Following a median of 4 cycles of treatment (range: 4–6 for VRD vs. 2–6 for VCD, P=ns), in the VRD-group 4 pts achieved complete remission (CR), 5 pts very good partial remission (VGPR ≥ 75% reduction of M-band) and 1 pt partial remission (PR: 50–75% reduction of M-band) while in the VCD-group CR:3, VGPR:2 and PR:3 pts (P=ns). The toxicities in terms of peripheral neuropathy, myelosuppression, liver and renal function were well tolerated and no patient discontinued treatment due to severe side effects. The 5-yr overall survival (OS) was 100% for the VRD-group vs. 75% for the VCD-group; nevertheless, the difference was not significant due to the size sample of the pt groups. The stage at diagnosis, the disease status pre-ASCT and the maintenance post-ASCT did not influence the OS. Interestingly, the 4-yr progression free survival (PFS) was significantly superior for patients who had been induced with the VRD regimen (75% vs. 36% P=0.05) and for patients who achieved CR or VGPR before ASCT (PFS: 50%) while no pts with PR pre-ASCT was progression-free 2 yrs post ASCT (P=0.001). In multivariate analysis, only the CR or VGPR status before ASCT favorably affected the long term PFS. Our results are in line with the limited published data from other studies with larger series of patients. In our study, very low disease burden before ASCT proven to be an independent factor for prolonged PFS. Taking into consideration that VRD resulted in more CR or VGPR status, it is reasonable to conclude that VRD is a highly effective regimen and could be first treatment choice for newly diagnosed MM patients who are fit for early ASCT post induction.

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#### P638

##### **Calcineurin inhibitor as graft versus host disease prophylaxis in patients with relapsed multiple myeloma undergoing allogeneic stem cell transplantation**

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Allogeneic stem cell transplantation (AlloSCT) is a potentially curative option for patients with Multiple Myeloma (MM). Despite the improvement of reduced-intensity-conditioning (RIC), transplant-related mortality (TRM) remains high. There is no consensus on which graft versus host disease (GVHD) prophylaxis regimen is superior. Some studies have suggested that Tacrolimus-based prophylaxis is more effective than Cyclosporine (Csa) in terms of lower incidence of severe acute GVHD (aGVHD), with no impact on overall survival (OS). Herein, efficacy and toxicity between two GVHD prophylaxis regimens is analyzed. We retrospectively analyzed 14 patients (pts) with relapsed MM who received AlloSCT RIC in the period from 2003 to 2015 in a single centre (Table 1). Population: age, 51 years (40–73); median follow-up: 19 months (1–175). Conditioning regimen: Allo-RIC (Fludarabine + Busulfan or Melphalan regimens) and 100% was Bortezomib-based in the Tacrolimus group. Donor: matched related (11 pts), unrelated (1), mismatch unrelated (1) and haploidentical (1) donor. GvHD prophylaxis: all patients received a short course of Methotrexate + Csa (9 pts, 64%) or Tacrolimus (5 pts, 36%). Complete response at transplant was 33% at Csa group and 60% at Tacrolimus group. All pts underwent toxicity related to chemotherapy (mainly mucositis and neutropenic fever) with organ impairment (renal or liver) in 100% Tacrolimus arm as well as 4 pts in Csa group. The incidence of aGVHD was 80% and 77.8% in Tacrolimus and Cyclosporine groups, respectively ( $P=0.99$ ). Grade III–IV aGVHD were reported in 2 pts (40%, Tacrolimus) and 4 pts (44%, Cyclosporine), with severe gastrointestinal and liver involvement. Glucocorticoid resistance was observed in 75% in both groups. Patients with refractory aGVHD received other immunosuppressive therapies: more than 3 second-lines agents (3–6) were necessary in fifty percent of pts in both groups to control GVHD. Two patients had to interrupt Tacrolimus due to neurological toxicity and suspected thrombotic thrombocytopenic purpura. No patients had to discontinue treatment in the Csa arm because of toxicity. The 12-months OS was 78.6% (80% in Tacrolimus vs 66.7% in Csa ( $P=0.78$ )) and the 24-months was 68.8%. A total of 4 pts died because of GvHD. During follow-up, only 2 patients relapsed (10 and 126 months after AlloSCT, respectively) in Csa group. No relapse were seen in Tacro group. In our experience, no significant differences were observed between both calcineurin inhibitor in terms of OS, toxicity and GVHD incidence. An explanation could be our small number of patients. AlloSCT is an effective therapy for selected patients but it is associated with high rates of GVHD and TRM. A long-term-safety and effective prophylactic regimen is necessary as main objective.

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	MTX + Tacrolimus	MTX + Cyclosporine
Age (median)	59	52
MM previous lines	3	2.2 (1-5)
Previous ASCT		
1	3	3
2	2	6
Disease status		
CR	3	3
PR	2	4
SD	-	1
E/E	-	1
RIC conditioning		
Bortezomib-based	5/5 (100%)	1/9 (11%)
CD34+ (median) $\times 10^6$ /kg	5.09	4.17
aGVHD	4/5 (80%)	7/9 (77.8%)
cGVHD	2/5 (40%)	4/9 (44%)
Glucocorticoid resistance	3/4 (75%)	6/8 (75%)
Treatment related Mortality	1/5 (20%)	4/9 (44%)
GvHD	1	3
Other causes	-	1 (IFI)

Disclosure of conflict of interest: None.

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### CD34+ cell dose is an independent prognostic factor in patients with Hodgkin lymphoma and multiple myeloma who underwent autologous stem cell transplantation

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Several parameters, including early lymphocyte, neutrophil, platelet recovery, and infused dose of CD34+ cells, have been associated with clinical outcome of patients with haematological malignancies. However, their prognostic significance remains uncertain. The aim of current study was to evaluate prognostic significance of clinical and laboratory parameters that might influence survival after autologous stem cell transplantation (ASCT) in Hodgkin lymphoma (HL) and multiple myeloma (MM). This retrospective study included a total of 90 with HL and 114 MM patients (median age 32 years, 55 years, respectively) who underwent ASCT between November 2005 and June 2016. HL patients were conditioned with BEAM (84.4%) and CBV (15.6%) regimen, while MM patients received conditioning with High dose of Melphalan. High IPS (International Prognostic score) at diagnosis had 68.9% HL patients and high ISS (International Scoring System) had 27.2% of MM patients, of which 8.8% had renal impairment. The average of transplanted CD34+ cells in HL patients was  $7.15 \times 10^6$ /kg (range:  $2-25.0 \times 10^6$ /kg), and  $6.6 \times 10^6$ /kg (range:  $2-15.51 \times 10^6$ /kg) in MM patients. After ASCT, favourable treatment response (partial/complete remission) achieved 83.3% HL patients, of whom 22.7% had infused  $< 5 \times 10^6$ /kg CD34+ cells. Median time to recovery of absolute lymphocyte count  $500 \times 10^6$ /l or greater (ALC500) was 16 days (range: 9–31 days), recovery of absolute neutrophil count  $\geq 500 \times 10^6$ /l (ANC500) was 12 (range: 6–26 days), and platelet recovery  $\geq 20 \times 10^9$ /l (PLT20) was 12 days (range: 5–44 days). After ASCT, 93.6% MM patients achieved favourable treatment response, of whom 26.5% had infused CD34+ cell dose  $> 8.7 \times 10^6$ /kg. Median time to ALC500 was 15 days (range: 9–23 days), ANC500 was 13 (range: 9–24 days), and PLT20 was 11 days (range: 5–26 days). Median follow up of patients with HL was 67 months, while after ASCT, median event free survival (EFS) was 20 months, and overall survival (OS) was 38 months. Treatment response after ASCT strongly influenced both EFS and OS after ASCT ( $P < 0.0001$ ). In patients who achieved favourable treatment response, OS and EFS after ASCT were influenced by infused CD34+ cell dose ( $< 5 \times 10^6$ /kg vs.  $\geq 5 \times 10^6$ /kg), prolonged recovery of ALC500 by Day+20, PLT by Day +13, and achieving of ANC500 by Day +11 ( $P < 0.05$ ). Multivariate analysis among significant variables showed that infused CD34+ cell dose was the most important parameter that influenced OS and EFS ( $P < 0.05$ ). Median follow up of MM patients was 50 months, while after ASCT, median EFS was 26 months and OS was 34 months. Regarding patients who achieved favourable treatment response, OS and EFS after ASCT were influenced by the presence of renal impairment, infused CD34+ cell dose ( $\leq 8.7 \times 10^6$ /kg vs.  $> 8.7 \times 10^6$ /kg) and PLT20 recovery by Day +13 ( $P < 0.05$ ). Among these significant parameters, multivariate analysis pointed out infused CD34+ cell dose as the most important parameter that influenced both OS and EFS ( $P < 0.05$ ). These data suggest that number of infused CD34+ cells is an independent factor that may contribute to outcome of patients with HL and MM.

Disclosure of conflict of interest: None.

P640

### Cost effective outcome of generic medicines used in CyBOR protocol induction and generic Melphalan Autologous transplant conditioning: Experience in 25 patients of multiple myeloma from single center in India

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High-dose therapy with Autologous Stem Cell Transplantation (ASCT) has become the treatment of choice for symptomatic eligible patients with Multiple Myeloma (MM). We studied an induction regimen of Cyclophosphamide, Bortezomib and Dexamethasone (CyBorD) and showed rapid and deep responses after 4 cycles in patients with newly diagnosed MM and we subsequently done ASCT with Melphalan (Mel) conditioning. Cost is the major limiting factor in developing world. All the drugs used are Generic brands manufactured in India. A total of 25 MM patients (median age: 54.5 years, 76% male and 24% female) were transplanted between 2012 and 2016. In all, patients had IgG kappa-12 (48%), IgG Lambda-03 (12%), IgA lambda-02 (20%), IgA kappa-02 (08%), Kappa light chain 02 (08%), Lambda light chain 01 (04%) patients. Prior to autograft, all cases had received CyBorD with generic medicines. Median time diagnosis to ASCT was 7.5 months (5 to 21 months). Stem cell Mobilization was done with G-CSF alone in 17 (68%), G-CSF plus Plerixafor in 07 (28%) and Chemo mobilization in 01 (04%) patients. All patients received ASCT support after conditioning with 200 mg/m<sup>2</sup> generic Melphalan alone (Dose adjustment was done according to renal status). All patients received Thalidomide maintenance from March 2012. Bortezomib used was manufactured by Dr. Reddy's lab, Hyderabad and Melphalan used was manufactured by Emcure Pharmaceuticals, Pune, India. 68 patients from 1999 to 2011 received Cyclophosphamide, Vincristine, Adriamycin and Dexamethasone (CVAD) protocol of originator medicines followed by originator Melphalan conditioning and ASCT (CVAD-Mel-ASCT). At the time of autograft, 21 (84%) of patients were in complete remission, 02 (08%) in partial remission, 02 (08%) very good PR. Median day of engraftment was 10 for neutrophils and 14 for platelet. Transplant related Mortality was 16% (4/25) out of which 2 died of infection and 2 deaths of cardiac events. The PFS and OS rates were 80% and 84% at median follow up of 18.6 months. Patients who were treated with CVAD-Mel-ASCT had EFS of 74% at 2yrs and 52% at 5yrs. Cost of Bortezomib showed significant difference, generic was 4000USD where as for originator drug was 20000USD for 4cycles of chemotherapy. Cost of Melphalan also showed difference with 450USD for generic and 2000USD for originator drug. Generic CyBorD showed excellent response rate and allows excellent stem cell collection and transplantation which can further consolidate response and improve outcome. CyBorD induction and Melphalan conditioning with generic medicines can be considered a standard regimen for transplant-eligible patients with newly diagnosed MM in resource constraint situation. Generic CyBorD-Mel-ASCT is more cost effective than Originator CVAD-Mel-ASCT. Generic medicines produced in India are of good quality and cost effective. This study needs long term follow up to assess survival parameters at a median.

**Disclosure of conflict of interest:** None.

#### P641

##### **Impact of genetic abnormalities after autologous and allogeneic stem cell transplantation in multiple myeloma**

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Risk stratification in Multiple myeloma (MM), currently based on cytogenetic abnormalities, is critical for long term counseling of transplant-eligible patients, and application of risk-adapted treatment algorithms to maximize clinical outcomes. We examined the FISH-based risk stratification in a homogeneously treated population of transplant-eligible

myeloma patients. From 129 patients, 113 samples were evaluated by FISH on isolated plasma cells. 104 patients were treated with Bortezomib, 45 patients received auto HSCT and 13 patients received allo HSCT. Patients were classified as High Risk (HR) if they had del(17p), t(14;20), t(14;16); and 1q abnormalities, as Standard Risk (SR) if they had t(11;14), t(6;14) and an extra copy of one or more odd-numbered chromosomes and as Intermediate Risk (IR) if they had t(4;14) or del(13)(q). Overall survival (OS) and relapse-free survival (RFS) were calculated from the time of Allo HSCT and Auto HSCT on day 0, from diagnosis to death or disease progression. The median age at presentation was 53.86 (range: 20–80) years, and 72 (63.7%) were men. At a median follow-up time of 18 months, 73% were alive. 45 of the 113 patients with available FISH samples underwent Auto HSCT. 24 patients (53.3%) achieved CR and 21 patients (46.7%) relapsed. Of the 13 patients who had received Allo HSCT, five patients (38.5%) achieved CR and five patients (38.5%) remained alive. In patients who received Auto HSCT, the risk of relapse was 56% less than those never transplanted ( $P=0.02$ ), but the difference was not significant in patients who received Allo HSCT. The relapse-free survival in HR patients was 6 months ( $P<0.001$ ), in IR was 11 months ( $P<0.001$ ) and in SR was 37.67 months ( $P<0.001$ ). In transplant patients, RFS in HR patients was 5.73 times more than SR group ( $P<0.001$ ) and in IR group was 3.35 times more than SR ( $P<0.001$ ). The survival time in transplant patients was significantly better than non-transplanted patients ( $P<0.001$ ). The median overall survival (OS) in HR patients was 25.45 months, in standard risk group 30 months and in SR patients was 31 months. Cytogenetic abnormalities detected by FISH are of significant value in classification, risk stratification and management of patients with MM. We can use cytogenetic data to provide prognostic information and also to guide management and clinical practice. These data indicate that autologous stem cell transplantation could potentially be of benefit to myeloma patients.

**Disclosure of conflict of interest:** None.

#### P642

##### **ISS stage and response to prior therapy are more important than maintenance therapy choice in post-transplantation multiple myeloma outcomes**

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Post-transplantation lenalidomide and bortezomib maintenance therapy for multiple myeloma (MM) have separately been shown to improve progression-free survival (PFS) compared to placebo but have never been directly compared for efficacy (McCarthy et al., 2012; Sonneveld et al., 2012). We performed a retrospective cohort study to investigate the efficacy of lenalidomide maintenance therapy compared to bortezomib maintenance in MM patients post-transplantation. 156 patients with MM treated at Vanderbilt University between 2004 and 2016 met inclusion criteria: 92 patients in the maintenance lenalidomide cohort and 64 in the maintenance bortezomib cohort. Baseline characteristics and outcome data were obtained via chart review. The primary outcome was PFS. The secondary outcomes were overall survival (OS) and treatment-related toxicities. The median follow-up time was 33 months. Median time to death (4.28 years vs 5.77,  $P=0.47$ ) and median time to progression (1.71 years vs 1.74,  $P=0.77$ ) were not significantly different in the maintenance lenalidomide cohort compared to the maintenance bortezomib cohort. In the multivariate analysis, PFS was worse in patients at International Staging System (ISS) stage 3 at diagnosis compared to those at ISS stages 1 and 2 (HR, 1.86; 95% CI, 1.11 to 3.12;  $P=0.02$ ) and worse in patients with less than Very Good Partial Response (VGPR) to last prior therapy compared to those with a response to prior therapy of at least VGPR (HR, 2.05; 95% CI, 1.14 to 3.69;  $P=0.02$ ) [see Figure 1]. PFS was improved in patients with more than two years of maintenance therapy compared to those with less than two

years of maintenance therapy (HR, 0.40; 95% CI, 0.22–0.70;  $P < 0.01$ ), but this result does not account for patients who ended maintenance therapy due to disease progression. OS was worse in patients at ISS stage 3 at diagnosis compared to those at ISS stages 1 and 2 (HR, 3.87; 95% CI, 1.44 to 10.39;  $P = 0.01$ ). Peripheral neuropathy was more common in the bortezomib cohort (39% vs 9%,  $P < 0.01$ ), while cytopenias were more common in the lenalidomide cohort (30% vs 3%,  $P < 0.01$ ).

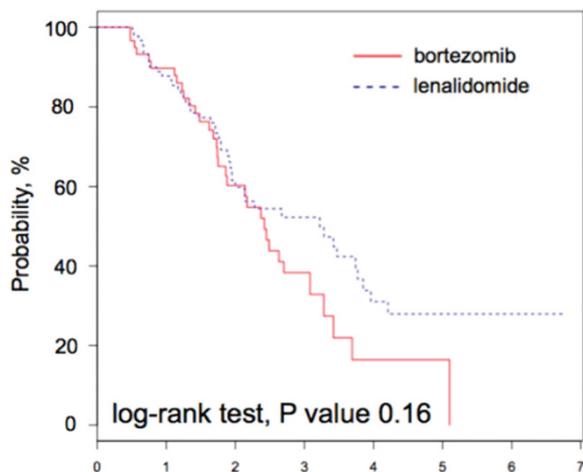
**Figure 1**

Kaplan–Meier curve for PFS for the maintenance lenalidomide group versus the maintenance bortezomib group by log-rank test ( $P = 0.16$ ).

Lenalidomide and bortezomib maintenance after transplantation have equal efficacy in prolonging progression-free and overall survival in patients with multiple myeloma. ISS stage significantly affects time to progression and overall survival, and response to last prior therapy affects time to progression. Length of maintenance therapy may be a significant predictor and warrants further analysis. These findings suggest that both lenalidomide and bortezomib are acceptable maintenance therapy options for post-transplantation multiple myeloma patients.

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**Disclosure of conflict of interest:** None.

**P643**

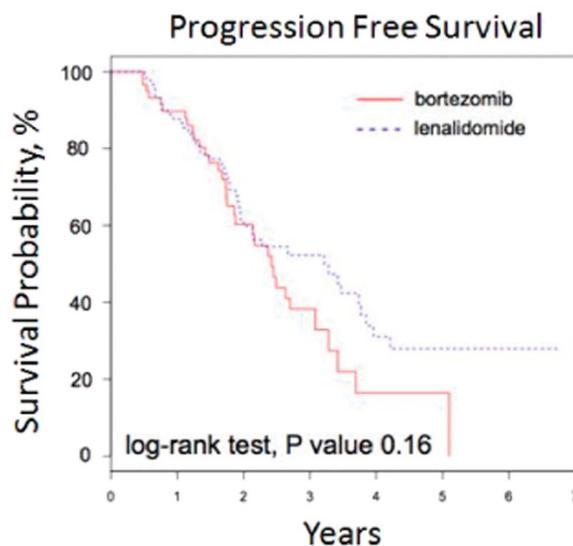
**Lenalidomide vs. bortezomib maintenance choice post-autologous haematopoietic cell transplantation (AHCT) for multiple myeloma**

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Post-AHCT lenalidomide (L) and bortezomib (B) maintenance therapy for multiple myeloma (MM) have separately been shown to improve progression-free survival (PFS) compared to placebo but have never been directly compared (McCarthy et al., 2012; Sonneveld et al., 2012). We performed a retrospective study to investigate survival outcomes and toxicities of L maintenance therapy compared with B maintenance in MM patients post-AHCT. This study included 156 patients who received AHCT for MM between 2008 and

2015 after induction with L- or B-based therapy. All patients received AHCT within 12 months of MM diagnosis and received melphalan 200 mg/m<sup>2</sup> conditioning. Patients who received tandem transplantations (autologous or allogeneic) were excluded. Only patients initiating maintenance therapy within 6 months post-AHCT were included. Maintenance therapy was defined as monotherapy with either L or B. The primary outcome was PFS. Secondary outcomes were overall survival (OS) and treatment-related toxicities. 92 patients received L maintenance and 64 B maintenance post-AHCT. At baseline there were no differences in ISS stage, DS stage or cytogenetic risk between maintenance cohorts. At time of analysis, 49% ( $n = 45$ ) receiving L maintenance and 52% ( $n = 33$ ) on B maintenance experienced disease progression. Median time to progression (1.71 vs 1.74 yrs,  $P = 0.77$ ) was not significantly different between cohorts. By multivariable analysis, choice of maintenance (L vs B) was not significant for PFS or OS. Variables significant for improved PFS were ISS stage I/II vs III (HR 1.86; 95% CI 1.11–3.12;  $P = 0.02$ ) and achieving at least very good partial response (VGPR) post-AHCT (HR 2.05; 95% CI 1.14–3.69;  $P = 0.02$ ) [see Image]. Patients continued on maintenance therapy for less than 2 years experienced progression earlier compared to greater than 2 years (HR 0.40; 95% CI 0.22–0.70;  $P < 0.01$ ). Disease response improved while on maintenance in 38% ( $n = 24$ ) with L and 34% ( $n = 31$ ) with B. Median OS was not statistically different between maintenance cohorts (4.28 vs 5.77 yrs,  $P = 0.47$ ). ISS stage I/II vs III resulted in improved OS (HR 3.87; 95% CI 1.44–10.39;  $P = 0.01$ ). Secondary malignancies occurred in 7% ( $n = 6$ ) with L and 3% ( $n = 2$ ) with B. Peripheral neuropathy was more common in the B cohort (39% vs 9%,  $P < 0.01$ ), while cytopenias were more common in the L cohort (30% vs 3%,  $P < 0.01$ ). The median follow-up time for survivors was 33 months. These findings suggest that both lenalidomide and bortezomib are equivocal maintenance therapy options for post-transplantation MM patients. Choice of maintenance therapy post-AHCT for MM did not demonstrate a difference in survival outcomes. Based on these data, maintenance choice should be guided by patient specific anticipated tolerance rather than drug type alone. ISS stage and post-AHCT disease response continue to be significant predictors for outcomes. Toxicities recorded on maintenance were as anticipated. Length of maintenance therapy may be a significant predictor and warrants further analysis. The analysis was underpowered to detect differences in outcomes based on cytogenetic features and should be explored with a larger dataset.

[P643]



**Disclosure of conflict of interest:** None.

**P644**

**Multiple myeloma: Allogeneic or autologous hematopoietic stem cell transplantation?**

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Autologous stem cells transplantation (auto-HCT) is an accepted method in multiple myeloma (MM) patients, but usually it is not curative. The issue of allogeneic hematopoietic stem cells transplantation (allo-HCT) is challenging yet for myeloma. We investigated allo-HCT in MM and compared with auto-HCT. In this retrospective study, we recruited 272 patients from January 2011 to January 2015 (218 (80.15%) patients in autologous group and 54 (19.85%) in allogeneic group). We performed allogeneic HCT with peripheral blood stem cells source in our center for patients who are relatively young (less than 55 years old) with good performance, have match sibling donor and accepted allogeneic HCT. The conditioning regimens in autologous group was Melphalan 200 mg/m<sup>2</sup> only and in allogeneic groups was Fludarabine 30 mg/m<sup>2</sup> plus Melphalan 140 mg/m<sup>2</sup> in 5 consequent days. GVHD prophylaxis consisted of Methotrexate and Cyclosporine. The outcomes then compared between two groups using log-rank and Gray tests and cox proportional hazard regression. The median follow-up in the autologous and allogeneic group was 17.02 months. Three years disease-free survival of auto-HCT

was 38.61% (CI: 27.37%, 49.72%) and 68.88% (CI: 50.74%, 81.47%) for allo-HCT patients (*P* value=0.0363). Three years overall survival of auto-HCT was 77.26% (CI: 66.08%, 85.16%) and 82.15% (CI: 64.92%, 91.44%) for allo-HCT patients (*P* value=0.6363) showing no significant statistical difference between two groups. Mortality rate was 11.01% for auto-HCT and for allo-HCT was 12.96%. The most common cause of death between two groups was relapse of primary disease. Three year relapse incidence was 20.83% (CI: 9.04%, 35.30%) for allo-HCT and 54.33% (CI: 42.02%, 65.09%) for auto-HCT (Gray's test *P* value=0.018). The three year TRM incidence was 10.36% (CI: 2.92%, 23.33%) and 7.01% (CI: 3.14%, 12.98%) in allogeneic and autologous patients respectively (Gray's test *P* value=0.42). Despite there was no statistically significant difference between two groups in terms of OS but DFS and relapse incidence was meaningfully better in allogeneic group. So, perhaps the reason of non-significant OS improvement in allogeneic group is higher early death due to higher TRM. We suggest that this study needs longer follow up to see whether allo-HCT resulted in OS improvement.

**Disclosure of conflict of interest:** None.

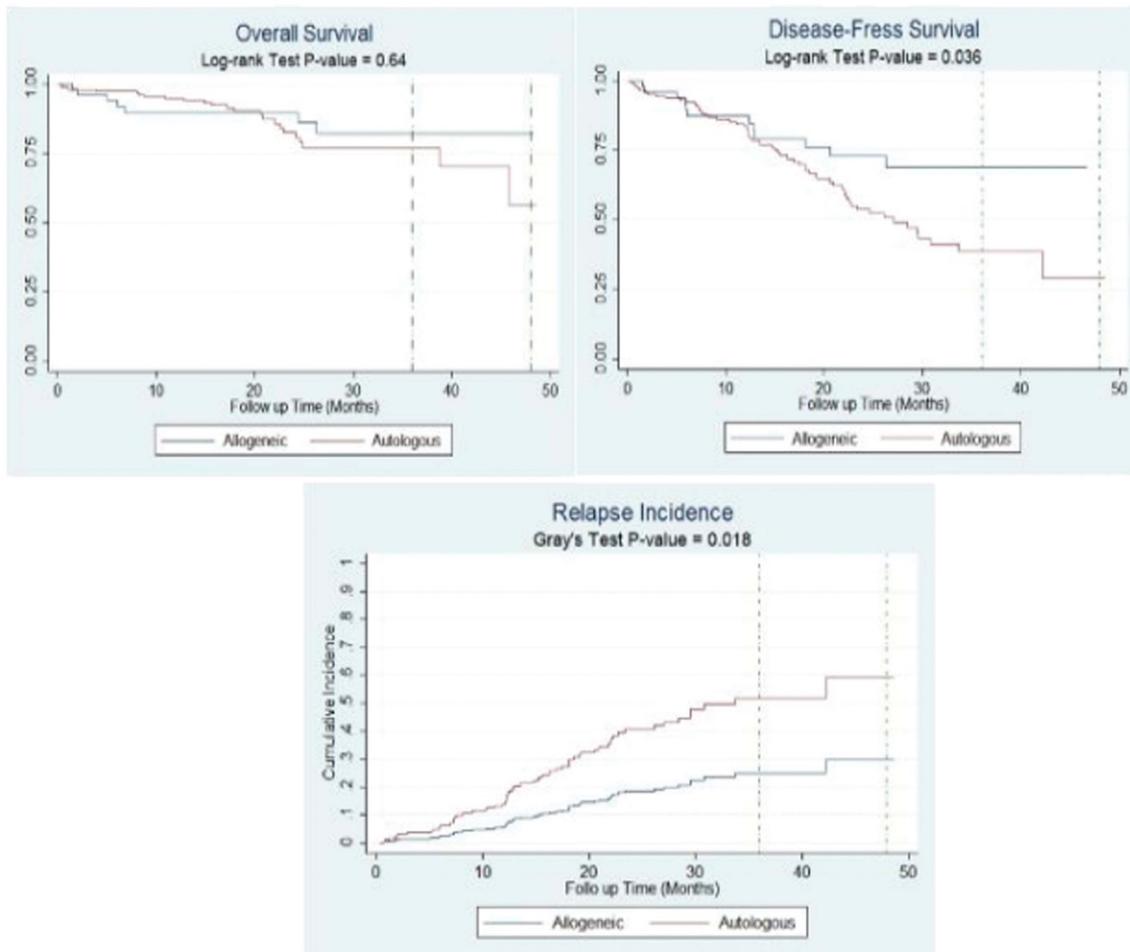
**P645**

**Myeloablative allogeneic hematopoietic stem cell transplantation from unrelated donors for patients with relapsed or refractory multiple myeloma**

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[P644]



Allogeneic hematopoietic stem cell transplantation (allo-SCT) for patients with multiple myeloma (MM) is increasing in number despite in the era of novel agents, especially as a second line treatment and beyond. It has been reported that allo-SCT for patients with MM resulted in high incidence of treatment related mortality (TRM). High incidence of disease relapse is also a major problem especially after reduced-intensity stem cell transplantation (RIST). It is an important issue to reduce the incidence of TRM while preventing disease relapse. The use of stem cells from unrelated donors is required for those without HLA-matched sibling donors. The purpose of this study is to evaluate the feasibility of an intensified conditioning regimen incorporating both 140 mg/m<sup>2</sup> of melphalan and 8 Gy of total body irradiation (TBI), followed by allo-SCT from unrelated donors for patients with relapsed or refractory MM. We retrospectively analyzed eight consecutive patients who received allo-SCT from unrelated donors with the conditioning regimen including 8Gy of TBI, fludarabine 25 mg/m<sup>2</sup> for five days, and melphalan 140 mg/m<sup>2</sup> between April 2013 and July 2015 at the Japanese Red Cross Medical Center. Six patients received unrelated bone marrow transplantation (BMT) and two patients received cord blood transplantation (CBT). Graft-versus-host disease (GVHD) prophylaxis was consisted of tacrolimus and short term methotrexate. The median age at allo-SCT, the time from diagnosis of myeloma to allo-SCT, and the numbers of prior treatment lines were 48.5 years (range: 31–60 years), 38.5 months (range: 8–64 months), and 3.5 lines (range: 1–7 lines), respectively. Five patients are female. No episode of either grade  $\geq$  III toxicity or non-relapsed mortality was documented during the median follow-up period of over two years. Cumulative incidence of grade  $\geq$  II acute and severe chronic graft-versus-host disease were 37.5% (95% confidence interval [CI] 7.2%–69.4%) at 100 days and 25.0% (95% CI 2.9%–58.1%) at 180 days, respectively. Probabilities of progression-free survival and overall survival were 22.5% (95% CI 0.0%–58.7%) and 58.3% (95% CI 22.0%–94.7%), at 3 years, respectively. The results suggest that allo-SCT conditioned with this intensified regimen may be tolerable for patients with relapsed or refractory MM.

**Disclosure of conflict of interest:** None.

#### P646

##### **Outcome of 66 allotransplants for myeloma at a single center: Severe acute GVHD and response inferior to CR are the two most critical factors for survival**

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The role of allogeneic stem cell transplantation (alloSCT) in the era of novel myeloma drugs remains controversial. It is the only curative treatment option but non-relapse mortality makes the decision making difficult as opposed to achievements with autologous SCT and new MM drugs by which the median survival is nowadays nearing 10 years. Aim of this study was retrospectively evaluate the outcome of alloSCT for MM performed at our institute, including evaluation of factors affecting survival. All 66 consecutive patients allotransplanted for MM between 1986 and 2014 were included. The data were collected from our transplant registry. Frequencies and medians were produced as appropriate. Kaplan–Meier method was used to calculate OS and PFS and log rank test for comparisons. Univariate analysis for factors affecting survival was performed with Cox proportional hazard model. Median age of all 66 patients was 55 (36–66) years. Half of the patients had IgG myeloma, 23% had ISS score 3 (score available for 30 patients), and 33% had high-risk cytogenetics (data available for 39 patients). Response to treatment at SCT was at least VGPR in 80% of patients, and transplant timing was early (within 15 months from Dg) in 58% of patients. Sibling donors

were used in 58% and MUDs in 42% of transplants, and conditioning was MA for 50% and RIC for another 50% of patients. AcGVHD grade 0–2 occurred in 78% and grade 3–4 in 22% of patients; 32% of patients had extensive chrGVHD. Post-transplant CR rate was 83%. 45% of patients have relapsed after alloSCT, and 42% are alive with the median follow-up of 5,6 years. Non-relapse mortality has been 30% (35% until 2005, 27% since then). The median survival of patients up to age of 60 years is 4.9 years vs 3.0 years for patients > 60 years ( $N=8$ ) with survival plateau after 6 years at 40% level. Transplant period, cytogenetics, donor type, conditioning intensity or occurrence of chrGVHD had no statistical impact on survival. Significant differences in OS were observed between disease status at SCR  $\geq$  VGPR vs < 15 months from Dg vs later), grade of acGVHD 0–2 vs 3–4, and best response post-transplant CR vs not less than CR. The respective differences for PFS were in SCT timing, grade of chrGVHD, and best post-transplant response. In univariate Cox regression analysis the only significant factors for OS were severity of acGVHD and CR vs other responses after SCT, and for PFS alloSCT timing, severity of chrGVHD, and best response to SCT. With alloSCT ca. 40% of MM patients can be cured but at the cost of high non-relapse mortality. The occurrence of grade 3–4 acGVHD and less than CR response to SCT predict poor survival. Considering the increasing survival expectations with modern standard therapy for MM, alloSCT may be recommended for younger patients with high-risk features, and alloSCT should be done early in the disease course.

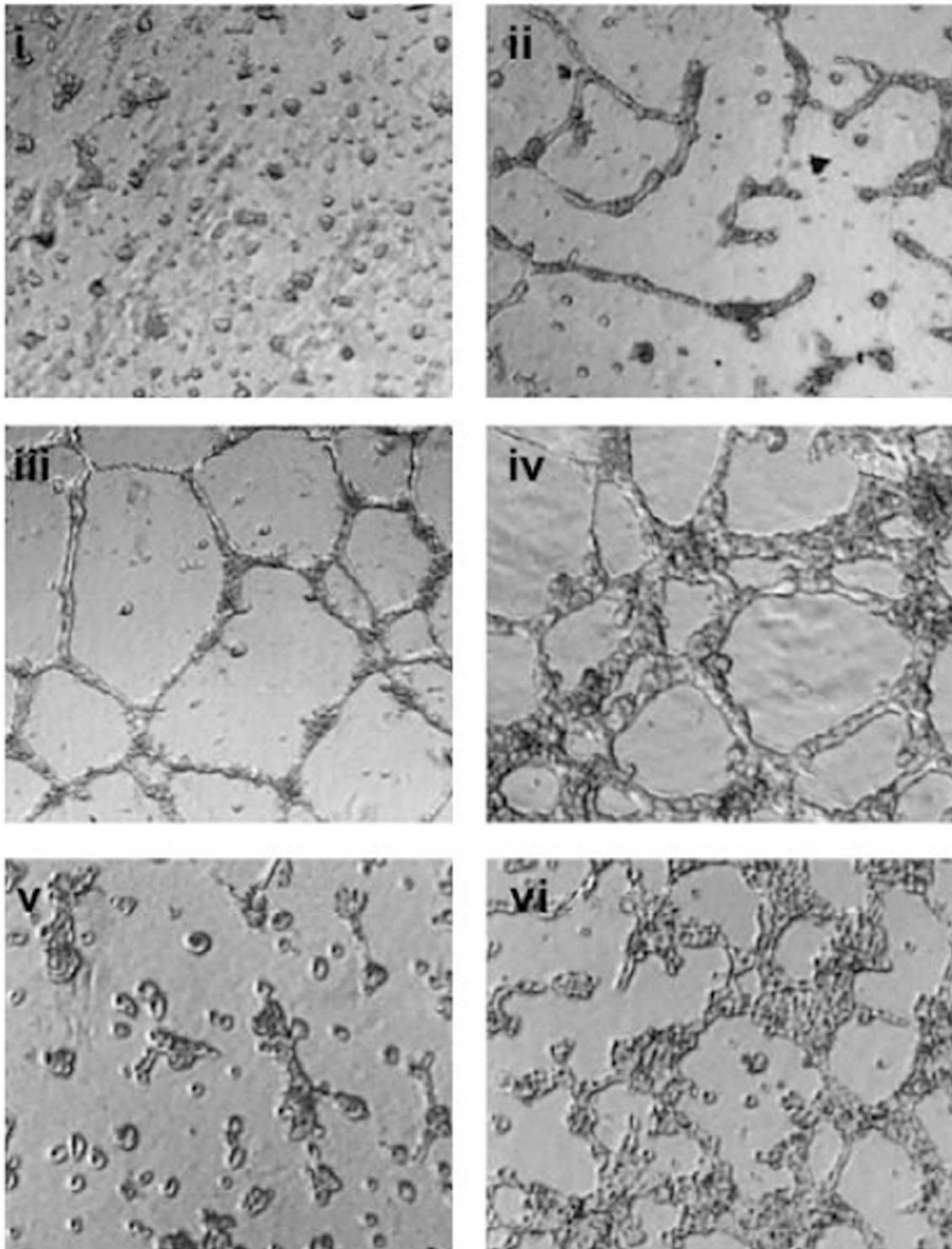
**Disclosure of conflict of interest:** None.

#### P647

##### **Overexpression of miR-21 involved in plasma cell myeloma-associated angiogenesis**

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Angiogenesis plays an important role in the pathophysiology of hematological malignancies including plasma cell myeloma (PCM). MicroRNA-21 (miR-21) is overexpressed and displays oncogenic activity in cancers. However, little is known about the role of miR-21 in PCM. The aim of the present study is to examine the expression level of peripheral miR-21 in PCM patients and to determine its role in angiogenesis. VEGF serum levels and miR-21 in PBMCs was measured in 93 patients with PCM directly before melphalan 200 mg/m<sup>2</sup> followed by autologous hematopoietic stem cell transplantation (auto-HSCT) and 2 months after HSCT; and 35 healthy controls. The study population was divided into two groups after therapy: responders (stringent complete response, complete response, very good partial response, partial response) and non-responders (stable disease, progressive disease). Gene expression of miR-21 was quantified by SYBR green real-time fluorescent quantitative PCR. Further tube formation of HUVECs and VEGF secretion was measured in miR-21 mimic or inhibitor transfected human plasma cell myeloma cell lines H929 and RPMI-8226. The expression level of miR-21 was significantly increased ( $2.7 \pm 0.55$  versus  $0.78 \pm 0.22$ ;  $P < 0.01$ ) in PBMCs of PCM patients compared with healthy controls. Further, serum VEGF levels were increased in PCM patients ( $477 \pm 145$  pg/ml versus  $178 \pm 78$  pg/ml in normal controls;  $P < 0.01$ ). After auto-HSCT, the expression level of miR-21 was significantly different in responders compared to non-responders. Responders had a lower expression of miR-21 compared to non-responders. Further, serum VEGF levels decreased in responders to auto-HSCT compared to non-responders. VEGF expression was increased in the supernatant from miR-21 mimic transfected human PCM cell lines H929 and RPMI-8226 compared with the negative control, while



VEGF was decreased in the miR-21 inhibitor transfected cell lines. The angiogenic ability of HUVECs was increased under pretreatment with the supernatant from H929 and RPMI-8226 cells transfected with miR-21 mimic compared with negative controls and decreased when pretreated with miR-21 inhibitor transfected cells (Fig. 1). This study demonstrated that miR-21 was upregulated in PCM patients. Responders to auto-HSCT had a decrease of miR-21 expression and VEGF levels. Further,

miR-21 regulated angiogenesis. Therefore inactivation of miR-21 or activation of its target gene may be a potential therapeutic approach in PCM. Fig. 1: *in vitro* Matrigel tube formation assay. (i), normal control (ii, iii), miR-21 mimic transfected H929 cells (iv), miR-21 mimic transfected RPMI-8226 cells (v), miR-21 inhibitor transfected H929 cells. Original magnification  $\times 100$ .

**Disclosure of conflict of interest:** None.

**P648**

**Pilot study of Busulfan/Thiotepa as conditioning regimen followed by allografting and post transplantation cyclophosphamide in advanced relapsed myeloma patients**

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Despite the significant improvement in outcomes has been observed for myeloma patients, the disease still remains incurable. Due to limitations, such as TRM and GvHD, the role of allogeneic stem cell transplantation as salvage therapy in this setting remains unclear. In present pilot study we provide data on the use of post Cyclophosphamide (ptCy) as GvHD prophylaxis after a busulfan/thiotepa based conditioning regimen in patients who relapsed after autologous stem cell transplantation. Between 11/2014 and 08/16 17 myeloma patients (male n=10, female n=7) with a median age of 55 years (range: 45–66) (pts), who relapsed after autologous stem cell transplantation received allogeneic stem transplantation with ptCy as GvHD prophylaxis after busulfan (9.6 mg/kg for age >60y and 6.4 mg/kg for age >60years) and thiotepa (10 mg/m<sup>2</sup>) and for haploidentical and MMUD additional fludarabine (90 mg/m<sup>2</sup>). All pts. were relapsed after one or two autologous stem cell transplantations. Donors were haploidentical (n=1), MMUD (n=4), MUD (n=6) and HLA-identical sibling (n=6). Stem cell source was PBSC (n=15) or BM (n=2). All patients received cyclophosphamide 50 mg/kg of body weight on day +3 and +4, which was in 10 pts (n=5 MRD, n=5 MUD) the only GvHD prophylaxis, while patients with MMUD and haploidentical donor received also cyclosporine A from day +5 and MMF (until day 35) and 2 patients (MRD and MUD) received additional cyclosporine. We observed no primary or secondary graft failure. The median time for neutrophil and platelet engraftment was 19 (range: 15–24) and 51 days (range: 22–279), respectively. Major toxicities grade 3 and 4 were: renal (n=1) and mucositis (n=1). Major infectious complications were: CMV: n=10 CMV-reactivations (n=10), sepsis (n=5), pneumonia (n=3) RSV-(n=2) and HSV (n=1). Acute GvHD grade II to IV and II/IV was noted in 29% and 24%, respectively and mainly seen in patients with cyclophosphamide as single GvHD prophylaxis. Remission rate were n=8 complete remission, n=7 vgPR, n=1 partial remission, n=1 n.a. After a median follow up of 12 months 3 pts progressed and 7 patients (n=1 relapse, n=6 TRM) died. The 1 year PFS was 18% (n=3). Busulfan/Thiotepa is an active conditioning regimen for advanced relapsed myeloma patients. Post cyclophosphamide might increase anti-myeloma activity, but as single GvHD prophylaxis it causes significant aGvHD in MRD and MUD and additional immunosuppressive agents such as cyclosporine should be added.

**Disclosure of conflict of interest:** None.

**P649**

**Quantitative analysis of T1-weighted MRI images as a surrogate for fat content does not correlate with response or survival**

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Magnetic resonance imaging (MRI) for multiple myeloma (MM) is a sensitive, non-invasive and non-toxic method for detecting

myeloma lesions. The goal of the study was to assess whether quantitative MRI metrics can detect treatment response and replacement of neoplastic cells by fat marrow. The study was HIPAA-compliant and IRB-approved. We retrospectively identified all patients who achieved a complete response (CR) after induction therapy between 2000 and 2014. Inclusion criteria for the study was total spine MRI imaging at diagnosis and after achieving CR. CR was determined using the IMWG criteria. Spinal vertebrae T12 through L5 were outlined with ImageJ software. Fractures and lesions were excluded. Images were analyzed using histogram-based (entropy, skewness, kurtosis) and texture-based statistics. A two-sided t-test was used to compare quantitative MRI metrics from before therapy and after achieving CR. Cox regression was used to explore the association between progression free survival (PFS) and change in each quantitative MRI metric based on a median split. PFS was defined as the time from the second MRI to death or progression of disease. Nineteen patients met the above criteria. Median age was 61.5 years (range: 37.5–72.2). Majority of patients (68%) were male. Majority of patients had ISS stage 1 disease (57.9%) and standard-risk cytogenetics (89.5%). An induction regimen containing an IMiD and/or a proteasome inhibitor was commonly used (73.7%). All patients received an autologous stem cell transplant (ASCT) consisting of high dose melphalan followed by autologous stem cell rescue. Three patients received a planned second ASCT. Seven patients (36.8%) were in CR before ASCT. Nine patients (47.4%) were treated with IMiD maintenance after planned initial therapy. Median time to repeat MRI imaging after CR was 10 months (range: 4.4–19.8). Mean change in measurements of kurtosis, skewness, entropy and 6 texture analyses are shown in Table 1. No significant change was detected between pre- and post-CR MRI. Furthermore, no significant association was seen between the change in any quantitative metric and PFS.

[P649]

Table 1

	Mean Change (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Skewness	-0.042 (-0.235 - 0.151)	0.65	0.9 (0.25 - 3.21)	0.88
Kurtosis	0.153 (-0.259 - 0.565)	0.44	2.38 (0.67 - 8.51)	0.18
Entropy	-0.001 (-0.004 - 0.002)	0.55	0.44 (0.11 - 1.73)	0.24
Texture T	-0.285 (-1.381 - 0.812)	0.59	0.66 (0.18 - 2.35)	0.52
Texture R	0.007 (-0.055 - 0.069)	0.81	0.75 (0.21 - 2.68)	0.66
Texture H	0 (-0.005 - 0.005)	0.99	2.16 (0.6 - 7.78)	0.24
Texture H	0.007 (-0.055 - 0.069)	0.81	1.35 (0.39 - 4.69)	0.64
Texture D	-0.008 (-0.147 - 0.13)	0.90	0.32 (0.08 - 1.28)	0.11
Texture S	-0.046 (-0.301 - 0.209)	0.71	0.66 (0.18 - 2.35)	0.52

Despite promising results by other groups, we could not find a significant association between quantitative T1 image analysis and CR or PFS. There was heterogeneity in the time of repeat MRI imaging which may have limited our ability to study interval change. Although no definitive conclusions can be made from this small sample, correlation between PFS and kurtosis or texture D may be promising and should be investigated in a larger group prospectively.

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**P650**

**Real-world multiple myeloma management practice patterns and outcomes in six central and Eastern European countries**

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Multiple myeloma (MM) treatment (Tx) has evolved in recent years. Solid data on the impact of new Tx on patient (pt) outcomes outside clinical trials, however, is lacking. This study aimed at investigating Tx practices, pt journeys, and outcomes in the real-world in countries with different access to new Tx. The study was conducted between 04/2015 and 06/2016 in Bulgaria, Croatia, Czech Republic, Poland, Romania, and Slovakia. It consisted of a cross-sectional (X) and a retrospective (R) phase. For the X-phase, investigators included all symptomatic MM pts seen during a 3-week counting phase to provide a snapshot of where in the pathway pts were at a given moment. For R-phase, investigators collected data on current and past Tx, including symptoms, dosages, administration schedule, Tx durations, Tx interruption, reasons for change/discontinuation, and Tx response. Pts were selected in reverse chronological order with a quota of a maximum of 3 pts who completed first-line (1L) Tx within the past 3 months (mo), 4 pts in second-line (2L) and 7 pts in third or higher lines (3+L). Pts included in the X-phase could also be included in the R-phase, if they met the respective inclusion criteria. In total, 39 physicians included 522 pts in the X- and 35 physicians included 277 pts in the R-phase. In the X- phase, 52% of pts were < 65, 36% were 65-75, and 12% were >75 years; the median time since diagnosis was 27 mo. 57% of pts were currently undergoing Tx, 41% were previously treated and 2% had never been treated. Of currently-treated pts, 37% received 1L, 30% 2L, 19% 3L and 14% 4+L. In the R-phase, 47% of pts were < 65 years. Of pts receiving 1L, 59% continued to 2L, 33% to 3L, 15% to 4L and 8% to 5L. Of the 38% of pts eligible for stem cell transplantation (SCT), 55% (=21% of all pts) received SCT at 1L; these proportions were similar across countries. The most frequently-used regimens in 1L and 2L were bortezomib-based (57% and 53%, respectively), in 3L and 4+L lenalidomide-based (47% and 35%, respectively). Median duration of 1L was 6 mo, followed by a median disease-free interval (DFI) of 4 mo. Median DFI was longer in pts with SCT than in those without (6.5 mo vs 1.5 mo). Time to progression (TTP) decreased with later Tx lines, from median 9 mo at 1L to 4 mo at 3L. Depth of response, as assessed by the treating physician, decreased with each additional line of Tx: 50% of pts achieved at least very good partial response ( $\geq$ VGPR) in 1L, while only 25% achieved  $\geq$ VGPR at 3+L. TTP was longer in pts with better response levels: in 1L, median TTP for pts with  $\geq$ VGPR was 22 mo versus 6 mo for pts with 6 mo for pts with < VGPR. The most common ( $\geq$ 20%, all grades) adverse events (AEs) and co-morbidities in 1L were anemia (42%), thrombocytopenia (29%), neutropenia (25%), neuropathy (25%), and fatigue (22%). These AEs disrupted treatment in 57% in 1L, 41% in 2L and 55% in 3+L. The study found that of SCT eligible pts, only slightly more than half were transplanted. Poorer outcomes and increasing AE incidence with each Tx line highlight the challenges of MM Tx. Information on real-world pt management may be valuable for physicians to plan their Tx strategies and can provide input for health economic evaluations of existing and new Tx.

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## P651

### Safety and efficacy of autologous stem cell transplantation in elderly patients with multiple myeloma

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Autologous stem cell transplantation (ASCT) is considered standard treatment for multiple myeloma (MM) patients under the age of 65 years, but its safety and efficacy still uncertain for patients over this age. Retrospective analysis from one single centre concerning MM patients under, equal or over 65 years who underwent ASCT between January/2010 and July/2016. It was also compared to 65–70 years old MM patients diagnosed in this period of time who were not transplanted. We analysed a total of 160 patients, 135 of which underwent ASCT. One-hundred-and-six of the transplanted patients were aged 65 years or less (median 56, IQR 10 years), 29 patients were aged more than 65 years (median 67, IQR 2 years) and 25 patients were non transplanted (median 68, IQR 4). The conditioning regimen for younger patients who underwent ASCT consisted mainly of melphalan 200mg/m<sup>2</sup>(MEL200) while half of the elder patients received melphalan 140mg/m<sup>2</sup> (MEL140). Regarding transplant-related myelotoxicity there were no statistical differences between patients aged 65 years or less and over 65 years old, however the first group needed less days of G-CSF ( $P=0.04$ ). Non-hematopoietic toxicity measured by infections and mucositis was not influenced by age. Patients >65 years conditioned with MEL200 had more days of aplasia ( $P=0.05$ ), greater need of G-CSF ( $P=0.01$ ) and transfusional support ( $P=0.04$ ) than patients  $\leq$ 65 years. There were no differences on non-hematopoietic toxicity. In the elderly group, patients conditioned with MEL200 presented more aplasia days ( $P<0.01$ ), higher grade of mucositis ( $P=0.03$ ) and more days of IV antibiotics ( $P=0.02$ ) than those transplanted with reduced dose of melphalan. Comorbidities had no effect on transplant-related toxicity, either by age or by dose of melphalan. Days of hospitalization and post-transplant complications did not differ according to age group. Transplant related mortality was 2% at day 100 post-transplant. Survival after transplant in patients 65 years old or under vs older patients (median follow-up time, 30 months), was not influenced by age (OS, 83mo vs 59mo,  $P=0.17$ ; PFS, 38mo vs 37mo,  $P=0.59$ ). Regarding the non-transplanted elderly group, these are patients with more renal disease ( $P=0.02$ ) and poorer performance status ( $P=0.04$ ) than the transplanted cohort. There is also higher cytogenetic risk ( $P=0.01$ ). Induction regimens were similar in transplanted group and non-transplanted group >65 years old, and response to first line therapy (before ASCT of transplanted group) revealed no differences. Infections were the most common complication in both groups. Transplanted patients needed less days of hospitalization ( $P=0.04$ ). Comparing the long term outcome of these two groups, survival curves of the elderly patients transplanted were clearly superior to the non-transplanted (OS, 62mo vs 21mo,  $P<0.01$ ; PFS, 45mo vs 20mo,  $P<0.01$ ) although one has to consider that the non-transplant group has worse features than the elderly transplant group. Transplantation in the elderly still debatable but this study shows that it might bring benefit. Globally, transplant related toxicity is not influenced by age. Regarding dose of melphalan, higher dose in elderly patients has higher toxicity, without apparent benefit in survival. Therefore, age should not restrict the access to ASCT, but instead selection must be based on individual clinical and functional status.

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**P652****Second autologous stem cell transplantation for relapse after allografting in multiple myeloma using CD 34+ selected donor cells without immunosuppression**

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Number of patients receiving a second allogeneic stem cell transplantation (SCT) in Europe is increasing despite high treatment related mortality (TRM). In multiple myeloma only very few reports of second allogeneic SCT exist with limited number of patients and substantial mortality. While in most hematological malignancies, the donor cell chimerism is dropping down if patients are relapsing, in myeloma donor cell chimerism remains complete despite relapse. To reduce TRM we thought that full donor cell chimerism may allow us to perform a second high dose busulfan based chemotherapy followed by "autologous transplantation" after stem cell mobilization and collection. However, because two consecutive patients failed to collect sufficient CD34+ cells for an autologous transplantation even with plerixafor, we used donor T cell depleted CD34+ selected cells and transplanted those patients in an "autologous" fashion without any immunosuppression. To enhance graft-versus-myeloma effect, we added donor lymphocyte infusion (DLI) at day 100. We report here on 11 myeloma patients with a median age of 58 years (range: 48–68) who relapsed after allogeneic SCT and underwent a second "autologous" SCT with CD34+ selected donor cells. All patients had received one ( $n=8$ ) or two ( $n=3$ ) autologous SCT before 1. allografting. 6 patients received an upfront auto-allo protocol and 5 patients received 1. allogeneic SCT as a salvage therapy. 73% of patients received a reduced intensity melphalan based conditioning regimen for 1. allogeneic SCT and the median PFS was 39 months (range: 22–56). Before 2. allograft patients had received overall a median of 5 (range: 3–7) treatment lines. At the time of 2. allogeneic SCT all patients had a full or nearly full donor cell chimerism and remission status was very good partial remission ( $n=1$ ), partial remission ( $n=5$ ), stable disease ( $n=4$ ), progressive disease ( $n=1$ ). 82% of patients received a myeloablative busulfan based conditioning regimen and all received CD 34+ selected stem cells with a median number of  $5.3 \times 10^6$ /kg CD34+ cells (range: 1.4–7.5) and  $5 \times 10^3$ /kg CD3+ cells (range: 1.6–6). Engraftment was noted in 100% at a median of 10 days (range: 9–14). No further graft-versus-host disease (GVHD) prophylaxis was performed and no acute GVHD (aGVHD) was observed. According to treatment plan, 9 patients received escalating DLI around day +100, starting with a median dose of  $2 \times 10^6$ /kg (range: 0.5–5) in combination with lenalidomide maintenance in 6 patients. 4 patients experienced aGVHD II–IV after DLI. Two patients had a severe GVHD (grade III) which resolved completely after steroid therapy. No nonrelapse mortality after SCT and DLI was observed. After a median follow up of 43 months (range: 6–49) the median PFS was 16 months (range: 8–24) which translates into a PFS for all patients of 61% at 1 year and 13% at 2 years. Median OS was 31 months (range: 13–48) and an OS of 69% at 2 years and 27% at 3 years was observed. For patients with advanced multiple myeloma relapsing after allografting, a second "autologous" SCT with CD34+ selected donor cells without immunosuppression followed by DLI is an encouraging treatment option with low toxicity.

**Disclosure of conflict of interest:** None.

**P653****Second autologous stem cell transplantation as treatment option for relapsed patients with multiple myeloma: A single center experience (CIC 859)**

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The use of modern therapies such as bortezomib, lenalidomide, thalidomide coupled with upfront high-dose therapy and autologous stem cell transplantation (ASCT) has resulted in improved survival in patients with newly diagnosed multiple myeloma (MM). The role of second ASCT as salvage therapy for relapse is unclear because of the availability of new agents to treat progression in multiple myeloma (MM). As the treatment options for management of patients with relapsed MM has become increasingly complex, physicians must consider both disease- and patient-related factors when choosing the appropriate therapeutic approach, with the goal of improving efficacy while minimizing toxicity. We retrospectively reviewed all MM patients who received a second ASCT as salvage therapy at our center from 2009 to December 2015. For this period we performed 211 transplants for MM patients. Twenty five (11.8%) patients received second ASCT (18 patients were relapsed) and for 7 patients ASCT was performed as tandem transplant. We analyzed only second ASCT for relapse. The median time to relapse after first transplant was 18.8 months (range: 8–50 months). All patients received reinduction therapy before the second ASCT. Conditioning was performed with Melphalan with two different doses ( $200 \text{ mg/m}^2$  and  $140 \text{ mg/m}^2$ ). The median age at second transplant was 51.8 years (range: 40–67 years), and female/man ratio was 4/14. Median interval between first and second ASCT was 28.3 months (range: 12–60 months). We have no observed early deaths. Until now 8 (45%) patients are dead because of progression disease. Response rate was assessed three months after ASCT, nine (50%) patients achieved VGPR, three (16.6%) patients achieved at least a partial response, three (16.6%) had SD and three (16.6%) progressed despite salvage ASCT. Median overall survival (OS) was 35.6 months (relapse  $\geq 24$  months = 47.7%;  $\leq 24$  months = 20). Second ASCT is a feasible and safe option for salvage therapy in MM, especially in Bulgaria where the possibility of using novel agents such as Carfilzomib, Lenalidomide, Daratumomab for relapsed patients is limited to clinical trials, because of no reimbursement. The best results were observed in patients whose time to progression was more than 24 months after first ASCT.

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**P654****Secondary malignancies in patients undergoing autologous hematopoietic cell transplant for multiple myeloma**

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Advances in treatment of multiple myeloma (MM) has improved overall survival in these patients (pts). A steady increase in the risk of secondary malignancies has been reported over the last decades in MM survivors. Estimated incidence of secondary acute myelogenous leukemia or myelodysplastic syndrome (t-MDS/AML) after treatment with alkylating agents is 1%–1.5% per year 2–10 years after primary chemotherapy. No specific risk factor has been recognized, but genetic instability, natural history of the disease as well as induction therapy and autologous stem cell transplantation (HCT) have been implicated. Recently, novel anti-myeloma treatments have been linked with an increase in secondary malignancies, but no solid relationship has been established yet. In a retrospective study, we analyzed the incidence of secondary malignancies (t-AML/MDS and solid tumors) in patients suffering from MM who had undergone autologous HCT using high-dose Melphalan conditioning regimen in our BMT unit. Study population consisted of 192 consecutive pts with median age of 55 years (29–70), 56.5% of them being male, who were transplanted during a period of 28 years (1988–2016). Type of myeloma was IgG/A/D in 56%, 18.8% and 0.5% respectively, while 17.2% was light chain and 7% non-secretory. The majority of pts presented with k light chain myeloma (62.8%). There was almost equal distribution between ISS stages I and II (45%/38.5%) and only 16.6% were diagnosed with advanced stage myeloma. Most pts received two lines of chemotherapy (60%) and all of them more than one. Treatment regimens before autologous HCT included VAD (63pts), Bortezomib-based (133pts), DCEP (8pts) and RD (29pts) and 34 pts received radiotherapy. Chemotherapy administration for mobilization was used in 18 pts (9.3%). Conditioning regimen before autologous HCT consisted of high-dose Melphalan (200 mg/m<sup>2</sup>) and in case of renal insufficiency 140 mg/m<sup>2</sup>. Incidence of a secondary malignancy was 5.7% after a median follow up period of 46 months. t-AML/MDS was diagnosed in 9 (4.68%) pts and 2 (1.02%) were diagnosed with breast and lung cancer respectively. Pts diagnosed with secondary malignancy were previously exposed in induction therapy to melphalan (6), VAD (3), Bortezomib (3), high-dose cyclophosphamide as mobilization treatment (4) and radiotherapy (4). Cytogenetic analysis was available in 6 patients diagnosed with t-MDS/AML and the majority (4/6) presented complex karyotype. Abnormalities mainly observed were deletions and insertions in chromosomes 5,7,17. Pts with secondary malignancies had an overall survival of 68 months (26–178), however, after malignancy diagnosis, survival was very poor, four months only (1–130). Secondary malignancies in pts with multiple myeloma after autologous HCT occur with a substantial frequency and have a dismal prognosis. The role of novel treatment agents has to be elucidated. Further studies are needed to identify new risk factors and develop better surveillance strategies.

**[P654]**

1	47, XY, del(12)(p12), -13, +mar
2	91, XYY, add(7)(q36), add(7)(q36), +8, +9, del(17)(p11), del(17)(p11), +mar1x2
3	45, XY, -4, del(5)(q13q33), t(7;17)(p14;p11), del(9)(q22), add(1)(q2;5)
4	45, XY, -7
5	43-45, XX, -5, del(7)(q32), -12, -13, add(17)(p13), -17, -18, +mar1, +mar2, +mar3
6	45, XY, -5,t(6;12)(p11;p13), del(7)(q31), del(17), t(17;?) (p13;?)

**Disclosure of conflict of interest:** None.

**P655****Survival analysis after allogeneic hematopoietic stem cell transplantation in patients diagnosed with multiple myeloma: A single center experience**

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) may provide long term remission cures for patients diagnosed with high-risk multiple myeloma. However, its use is limited since it has a high rate of treatment-related mortality (TRM), and because its efficacy compared to autologous HSCT is not fully established. We studied 16 patients that underwent alloHSCT between 2002–2015. Population characteristics are in Table 1. All patients were treated at least with one prior therapy lines (1–5), all including autoHSCT (43.75% underwent 2 prior autoHSCT). 83% had 2 or 3 prior therapy lines. 11 of them received bortezomib as part of treatment regimens. Donor characteristics: 2 non-related; 14 HLA-identical. GVHD prophylaxis: methotrexate plus a calcineurine-inhibitor: 12 cyclosporine and 4 tacrolimus. Median follow-up 15.5 months (1.3–174.5), average was 35.9 months. Seven patients died (43.75%); 2 because of progression (12.5%), and 5 (31.25%) due to TRM, including infections and immediate complications of transplantation, such as toxicities, ICU admission and aGVHD: Infections: 7 CMV reactivations, 3 invasive fungal and 7 bacterial infections. Disease status: 6 patients were in CR prior to alloHSCT. 3 of them maintained it after. Remaining patients died before disease was evaluated. Seven patients were in PR prior to transplant, and 4 reached CR after alloHSCT. One had progressive disease and reached CR after the procedure. Two had stable disease and progressed after allo; one of them is in CR after additional therapy lines, and the other one died 4 months after due to it. Donor characteristics: HLA-identical sibling donors: 87.5% (1 HLA-mismatch, passed away 2.7 months after allo due to TRM). One of the non-related donors, had an HLA-mismatch, and died 4 months after alloHSCT due to TRM, the other one is alive after 21 months. GVHD: 10 (62.5%) developed aGVHD and 3 of them maintained it chronically. Two suffer from cGVHD, plus 3 that initiated it as aGVHD. 9 were refractory to steroids. Long-term survivors: 3 patients had overcome three years after alloHSCT. They were among 39 and 50 years old at the time transplant was performed. None of them received bortezomib as part of therapy protocols for the disease. All had 2 therapy lines prior allograft. 2 were submitted to 2 prior autologous HSCT. Relapse: 4 patients relapsed after alloHSCT (25%, median time to relapse 6.2 months), being alive 50% at the end of the study. Allogeneic HSCT is associated with a high incidence of NRM and a low incidence of relapse. Rates of acute and chronic GVHD are high. In our cohort, besides that more than 50% are alive until now, they suffer from extensive chronic GVHD and are in need of treatment. Long-term

DISEASE STATUS AT ALLOHSCT	COMPLETE RESPONSE (37.5%)	PARTIAL RESPONSE (43.75%)	STABLE DISEASE (6.25%)	PROGRESSIVE DISEASE (12.5%)
Age (median)	57	48	50	41
MM previous lines	2.6	2.7	2	2
Previous ASCT	1 ASCT: 5 2 ASCT: 1	1 ASCT: 3 2 ASCT: 4	1 ASCT: 1 2 ASCT: 0	1 ASCT: 0 2 ASCT: 2
Complete remission post alloHSCT	50%	57%	0%	50%
Mortality	50%	43%	100%	50%

survival may be related with patient factors such as young age, but also low tumor burden, or less prior therapy lines; in our group there are no differences in this aspect. Studies including high-risk abnormal cytogenetics should help to define which patients are best candidates to alloHSCT.

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**Disclosure of conflict of interest:** None.

#### P656

##### The impact of melphalan dosage on outcomes of autologous haematopoietic cell transplantation for multiple myeloma: Results from the EBMT collaboration to collect autologous transplant outcomes in lymphoma and myeloma (CALM) study

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High-dose melphalan followed by autologous haematopoietic cell transplantation (AHCT) remains the standard of care for eligible multiple myeloma (MM) patients. The majority of patients in clinical practice and trials receive a melphalan dose of 200 mg/m<sup>2</sup> (MEL200), but a reduced dose of 140 mg/m<sup>2</sup> (MEL140) is often used in patients perceived to be unable to tolerate MEL200. It remains to be determined whether this considerable dose difference results in different clinical outcomes. We therefore analysed 1978 patients with MM who underwent a first single MEL140 or MEL200-conditioned AHCT between January 2008 and December 2012. All patients were included in the CALM study, an analysis of a prospectively defined cohort of patients with data reported retrospectively to the EBMT, covering AHCTs for MM and lymphoma. Patients in the MEL140 group were older than patients in the MEL200 group at the time of AHCT (median 64 years [range: 28–73] vs 59 years [25–76];  $P < .001$ ). Compared to the MEL200 group ( $n = 1733$ , 87.6%), fewer patients in the MEL140 group ( $n = 245$ , 12.4%) were overweight or obese (49.5% vs 63.9%;  $P = .003$ ). Compared to the MEL200 group, more patients in the MEL140 group had received proteasome inhibitor-containing induction therapy (71.7% vs 57.5%;  $P = .001$ ), had a Karnofsky score of  $\leq 80$  (38.2% vs 28.1%;  $P = .002$ ), and were transplanted in less than PR (13.0% vs 7.8%;  $P = .025$ ). Overall survival (OS) from the time of AHCT was not significantly different between the MEL140 and MEL200 group (6-year probability of OS 56.4% [95% CI 47.0–65.7] vs 55.1% [51.7–58.4];  $P = .77$ ), and neither was progression free survival (2-year probability of PFS 57.5% [51.2–63.9] vs 54.0% [51.5–56.4];  $P = .54$ ). Cumulative incidence of relapse (CIR) was not significantly different between the MEL140 and MEL200 group (2-year CIR 39.9% [33.7–46.2] vs 44.4% [42.0–46.8];  $P = .32$ ), and neither was non-relapse mortality (2-year NRM 2.5% [0.5–4.6] vs 1.6% [1.0–2.2];  $P = .26$ ). The main cause of death within 12 months of the transplant was relapse/progression in 77.8% of patients in the MEL140 group and 78.0% of patients in the MEL200 group. Second primary malignancy rates 5 years post AHCT in the MEL140 group were similar to the MEL200 group (4.8% [1.1–8.5] and 4.8% [3.6–6.0];  $P = .62$ ). In the MEL140 group, 54.4% of patients achieved a CR, compared to 61.3% in the MEL200 group ( $P = .044$ ). Median times to neutrophil and platelet recovery were not

significantly different between MEL140 and MEL200 (12 days in both groups for neutrophil recovery; 16 vs 15 days for platelet recovery). However, late neutrophil recovery was noted in a small proportion of patients in the MEL200 group. Neutrophil recovery > 21 days post AHCT was not observed in any engrafting patient in the MEL140 group, but occurred in 37 (2.2%) engrafting patients in the MEL200 group ( $P= .011$ ). A Cox proportional hazards model that included melphalan dose, age, and remission status at AHCT showed that melphalan dose had no effect on OS, PFS and relapse risk. The findings suggest that MEL140 is not inferior to MEL200 in younger and older MM patients and may reduce the risk of delayed haematological recovery in some patients. Further analyses in relevant subgroups such as patients with high-risk features or renal impairment are required.

**Disclosure of conflict of interest:** None.

#### P657

### The outcome of high-dose chemotherapy and autologous stem cell transplantation in transplant-eligible patients with multiple myeloma: A true story

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High-dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) remains the standard of care for patients younger than 65 years of age with multiple myeloma (MM). Different agents are being used to control the disease before ASCT, including the older thalidomide based combination or the newer bortezomib and lenalidomide based combination. The relation between the initial induction regimen and outcomes after ASCT is not completely clear. To evaluate the effect of different induction regimens on ASCT outcome, we retrospectively evaluated the outcomes of a low cost older regimen of Thalidomide based combination in doublets or triplets with newer novel agents like Bortezomib or Lenalidomide based combination in a low resources country in transplant-eligible patients with multiple Myeloma who underwent autologous stem cell transplantation at King Hussein Cancer Center BMT program. We retrospectively reviewed the files of patients diagnosed with MM from January 2008 till December 2015, who received induction treatment followed by HDT and ASCT and followed up in a single institution. We compared the effects of different induction regimens, disease stage, and remission status before transplantation on over-all survival (OS), event free survival (EFS) and progression free survival (PFS) using Kaplan Meier curves. A total of 94 patients were included, 54 (57.4%) of them received thalidomide based induction (group 1) and 40 (42.6%) received Bortezomib and Lenalidomide based induction (group 2). Patients also offered no consolidation nor maintenance therapy. 35 (37.2%) patients were stage I, 34 (36.2%) stage II and 15 (16%) were stage III. Stage was not documented for 10 (10.6%) of cases. 58 (61.7%) were in complete remission (CR) and 36 (38.3%) were in partial remission (PR). The estimated 5-year OS for the whole group was 57.7%. There was no statistically significant difference between both groups in regards to initial ISS stage of disease ( $P=0.332$ ) or CR status before ASCT. 32 patients (59.3%) in group 1 achieved complete remission (CR) or very good partial response (VGPR), while 25 (62.5%) patient in group 2 achieved CR or VGPR. There was no statistically significant difference between group 1 and group 2 in 5-years OS (5-year OS was 60% vs 57%,  $P=0.5007$ ), EFS (39.6% vs 52.6%,  $P=0.8029$ ) or PFS (45.2% vs 57.8,  $P=0.8033$ ). The use of an old, low-cost, Thalidomide-based regimen in a low-resources country achieved a favorable transplantation outcomes in patients with multiple myeloma who received HDT and ASCT.

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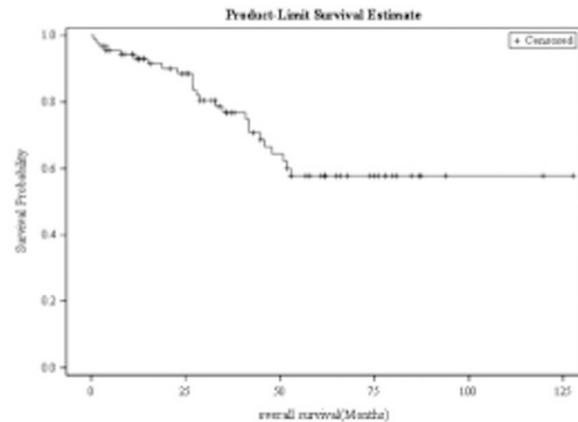
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#### P658

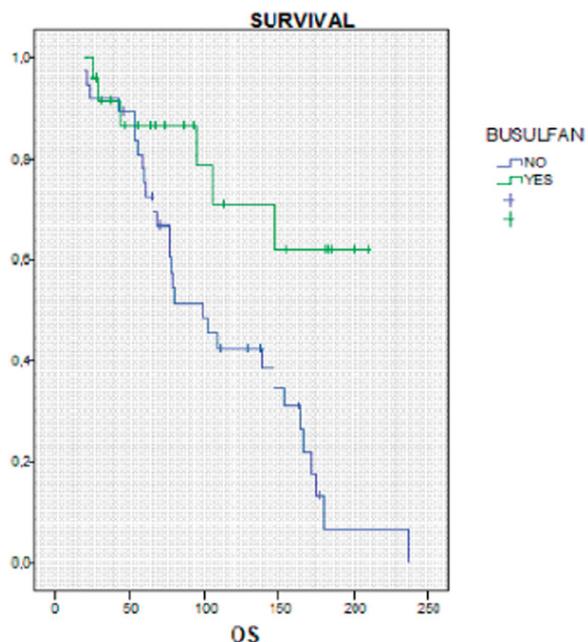
### When is the best moment, and which is the best conditioning regimen to use, for the second autologous transplantation in multiple myeloma patients?

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Double autologous stem cell transplantation (ASCT) is a useful treatment for Multiple Myeloma (MM) patients. We can make the second ASCT (2ASCT) without reinduction treatment (TANDEM regimen) or after a reinduction treatment after first ASCT (1ASCT) relapse (SALVAGE regimen). We have conducted a retrospective study over 61 MM patients undergoing a double ASCT performed in our centre from 1996 to 2016. We have compared the different conditioning regimens used, and if there are any difference between TANDEM or SALVAGE ASCT. We do not use maintenance treatment systematically. Characteristics of patients and conditioning regimens in Table 1. The overall survivals (OS) of our patients are 139 months (m) from treatment start till last control. The most important prognostic factors are the duration of the progression free survival (PFS) after 1ASCT (HR: 0.96 (0.94–0.99);  $P=0.006$ ), and the use of BUMEL like conditioning regimen at the 1ASCT or at the 2ASCT vs another conditioning regimens (HR: 3.43 (1.4–8.39);  $P=0.007$ ). Today there are 27 patients alive (43%), but only 10 (37%) are free of MM now. The 25 patients who were treated with TANDEM have a little better OS than SALVAGE patients (166m vs 103 m;  $P=0.55$ . Not significant). Patients at TANDEM group who received different conditioning regimen at the 1ASCT and at the 2ASCT live more time than patients treated only with MELPHALAN 200 (MEL200) at both ASCT. At SALVAGE group the duration of PFS after 1ASCT is better than the PFS after 2ASCT (28 m vs 13 m). The use of the same conditioning regimen at the both ASCT has worse results than if we use different treatment. Patients who were treated with

**Table 1. Characteristics and conditioning regimen used**

N° patients	Age					Alive	OS	
61	52 (37-68)					27	139 months	
Conditioning Regimen		SALVAGE (36)				TANDEM (25)		
	N°	N°	PFS1	PFS2	OS	N	PFS2	OS
MEL200+ CBV	14	6	44 m	5 m	77m	8	34m	166m
MEL200+MEL200	22	15	26m	9m	99m	7	16m	77m
MEL200+BUMEL	16	13	28m	19m	NR	3	17m	NE
BUMEL + CBV	7	1	5m	39m	106m	6	18m	NR
BUMEL + MEL200	1	1	105m	49m	186m	0		
MEL200+ MEL 100	1	0				1	17m	60m



BUMEL at the 1ASCT or 2ASCT have better OS than patients treated with CBV or MEL200. Patients who not responded to reinduction treatment before 2ASCT have worst PFS after 2ASCT (RC:29 m, Response; 19m and NOT response; only 10 m). Attention is drawn to the fact that patients who received BUMEL at 1ASCT have large OS, but they are very few (8) patients. Only one patient has died during the 2ASCT, and was a patient of SALVAGE group treated with BUMEL. Double autologous transplantation continues to be a useful treatment despite the new MM treatments, and allows to prolonged the OS. TANDEM ASCT probably is a useful treatment in high risk MM patients. SALVAGE treatment is most useful in patients with a large PFS after 1ASCT, and good response to reinduction treatment. Although MEL200 continue to be the standard conditioning regimen for ASCT in MM patients, we have observed that patients treated with different conditioning regimen at 1ASCT and 2ASCT have better prognostic, and BUMEL has the best results in our serie.

**Disclosure of conflict of interest:** None.

## Myelodysplastic Syndromes

### P659

#### Allogeneic haematopoietic stem cell transplantation for elderly patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML): A single center analysis

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Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The prognosis of elderly patients with MDS and AML after chemotherapy is poor. Allo-HSCT is feasible in these patients; however the management of elderly patients with MDS and AML for allo-HSCT is difficult. We performed a retrospective survey of allo-HSCT for elderly patients with MDS and AML in our institution. We retrospectively analyzed the records of elderly patients ≥ 60 years with MDS and AML who underwent allo-HSCT in our hospital between January 2011 and December 2015. In this study, we assessed the IPSS-R (Revised International Prognosis Scoring System) cytogenetic score and the IPSS-R score against the outcome of elderly MDS and AML patients who treated with allo-HSCT. Fifty-one elderly patients with MDS and AML were treated with allo-HSCT in our institution, 47 patients with MDS (29 with MDS overt AML) and 4 with de novo AML. Ages ranged from 60 to 71 years (median 64), 18 patients were female and 33 were male. There was a history of malignant disease in 14 patients. According to the IPSS-R cytogenetic scores of MDS patients, 10 patients fell in the good risk group, 7 were in the intermediate risk group, 7 were in the poor risk group, and 23 were in the very poor risk group. Regarding the IPSS-R score, 1 patient fell in the low risk group, 5 in the intermediate risk group, 6 in the high risk group, and 35 in the very high risk group. Sixteen patients were in 1st complete remission (CR), 1 patient was in 2nd CR, 9 patients were in partial remission, and 25 patients were not in remission (NR) upon administration of allo-HSCT. All patients received a reduced intensity conditioning regimen. 45 patients

were treated with fludarabine (Flu), melphalan and low dose TBI-containing regimens; 5 patients were treated with Flu, intravenous busulfan and low dose TBI; and one patient was treated with Flu, cyclophosphamide and low dose TLI. Graft-versus-host disease (GvHD) prophylaxis consisted of tacrolimus plus methotrexate in 46 patients, and tacrolimus, methotrexate and mycophenolate mofetil in 5 patients. Thirty-four patients received anti-thymocyte globulin (ATG). The donor source was sibling bone marrow (BM) in 1 patient, sibling peripheral blood stem cell in 7, unrelated BM in 36 and unrelated cord blood in 7. Relapse-free survival (RFS) and overall survival (OS) were 40.7% (95% confidence interval (CI): 27.2–53.8%) and 49.7% (95% CI: 35.1–62.7%) at 1 year, 31.4% (95% CI: 18.2–45.5%) and 33.6% (95% CI: 19.2–48.5%) at 3 years, respectively (Figure 1.). In this study, 4 patients died before engraftment. Non-relapse mortality (NRM) was 19.6% at day 100. Twenty-five patients developed chronic GvHD (3 patients limited and 22 extensive). The causes of death were disease progression (10 patients), treatment-related mortality (13 patients), infection (4 patients) and other causes (3 patients). We suggest that many elderly allo-HSCT patients with MDS and AML were in the very poor risk group when the IPSS-R cytogenetics score was assigned, in the very high risk group when the IPSS-R score was assigned and NR upon administration of allo-HSCT. RFS and OS were 31.4% and 33.6% at 3 years, respectively. There is a need for novel treatment strategies to manage elderly MDS and AML patients for allo-HSCT.

[P659]

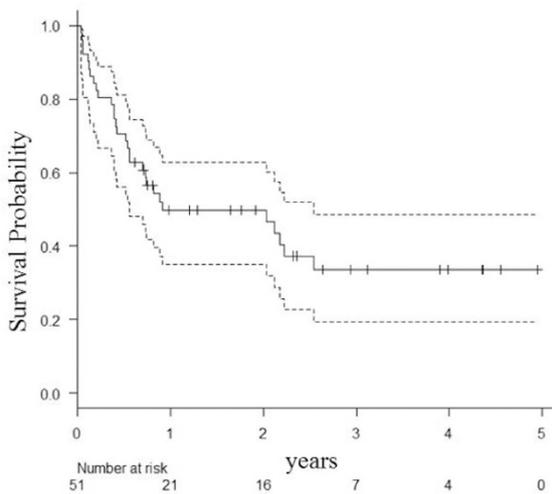


Figure 1. Kaplan-Meier estimate for overall survival.

**Disclosure of conflict of interest:** None.

#### P660

##### Counting bone marrow blasts as a percentage of non-erythroid cells provides superior risk stratification for MDS patients with erythroid predominance

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Patients with erythroid predominance ( $\geq 50\%$  erythroblasts, MDS-erythroid) compose a significant proportion of patients with MDS. The erythroid/myeloid subtype was divided from the AML category into MDS-erythroid by the 2016 WHO classification of myeloid neoplasms. At that time, there was no consensus on a more appropriate way of enumerating bone marrow (BM) blasts from TNCs or NECs in MDS-erythroid patients. To clarify these questions, 1283 MDS patients were

retrospectively analyzed in our center. MDS-erythroid was observed in 27.0% of patients (346/1283), and these patients had similar clinico-pathological features and overall survival, with 937 cases of MDS with  $< 50\%$  ENC. By calculating the percentage of BM blasts from NECs, 73 of 200 patients (36.5%) with MDS-erythroid who were diagnosed within WHO subtypes without excess blasts (EB) were moved into higher-risk categories and showed shorter OS than those who remained in the initial categories ( $P=0.041$ ). Recalculating the International Prognostic Scoring System-Revised (IPSS-R) by enumerating blasts from NECs, 40 of 168 (23.8%) MDS-erythroid patients with relatively lower risk were re-classified as higher-risk and had significantly poorer survival than those who remained in the lower-risk category ( $P=0.030$ ). This was especially true for the intermediate risk group that was stratified by IPSS-R (unchanged patients vs. shifted patients,  $P=0.007$ ). However, the impact of enumerating BM blasts from NECs on classification and prognostication was not evident in all MDS patients. In conclusion, our results suggested that enumerating the percentage of BM blasts from NECs significantly improved the prognostic assessment of MDS-erythroid, especially for patients within the intermediate risk group stratified by IPSS-R.

**Disclosure of conflict of interest:** None.

#### P661

##### Effect of CD34+ cell dose on outcome of allogeneic hematopoietic stem cell with myelodysplastic syndrome in children and adolescents

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Myelodysplastic syndrome (MDS) is a group of clonal and heterogeneous diseases, characterized by ineffective hematopoiesis. The incidence of MDS is about 5% of all blood disorders in children, approximately 40% of them develops acute leukemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is effective curative treatment of MDS in children, but depends on disease status, type of clonal chromosomal abnormalities presented at the time of allo-HSCT and graft quality. The aim of this study: To analyze the influence of graft quality on the outcome of childhood MDS after allo-HSCT. Allo-HSCT were performed in 58 patients (pts) (39 boys; 19 girls) with following MDS types: refractory cytopenia of childhood—11 (19%), refractory anemia with excess blasts -14 pts (24%), refractory anemia with excess blasts in transformation—22 pts (38%), juvenile myelomonocytic leukemia in 11 pts (19%). The median of age was 7 years (1–19 years). Unrelated allo-HSCT was done in 39 pts (67%) pts, related—in 10 pts (17%) pts, haplo- in 9 pts (16%). Myeloablative conditioning regimens (MAC) were used in 30 pts (52%); reduced-intensity conditioning (RIC) in 28 pts (48%). MAC consisted Busulfan (Bu) 16 mg/kg + Cyclophosphamide 120 mg/kg. RIC included Fludarabine (Flu) 150 mg/m<sup>2</sup> + Melphalan (Mel) 140 mg/m<sup>2</sup>, Flu 150–180 mg/m<sup>2</sup> + Bu 8mg/kg. The bone marrow (BM) was used in 38 pts (66%), peripheral blood stem cells (PBSC) in 13 pts (22%), combination of BM and PBSC in 7 pts (12%). 5-years overall survival (OS) was 45%. Engraftment was achieved in 42 pts (72%). OS after MAC allo-HSCT—49%, after RIC allo-HSCT—42% ( $P=0.27$ ). OS in group of RCC 50%; RAEB—44%, JMML—47%, RAEB-T—40% ( $P=0.103$ ). OS was in PBSC group -28%; BM group—50%, combination of BM and PBSC—25% ( $P=0.103$ ). At CD 34+  $\leq 5.0 \times 10^6$ /kg cell dose OS was 37%, CD 34+  $\geq 5.0 \times 10^6$ /kg—49% ( $P=0.047$ ). Dose of CD34+  $\leq 5.0 \times 10^6$ /kg recipient weight was associated with higher transplant related mortality (39% versus 10%,  $P=0.031$ ). Our data demonstrate that graft quality reliably influence on OS with childhood MDS.

**Disclosure of conflict of interest:** None.

**P662**

**Hypomethylating agents vs. Allogeneic sct in elderly patients with advanced myelodysplastic syndromes: A single center study**

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A group of 26 patients older than 50 years of age with myelodysplastic syndrome (MDS) RAEB II or with acute myeloid leukemia with multilineage dysplasia with less than 30% of bone marrow blasts (MDS RAEB-T according to the FAB classification) was treated with hypomethylating agents (HMA) and the results were compared to those obtained in an age and diagnosis matched group of 16 patients who underwent allogeneic stem cell transplantation (SCT). In the HMA group, 22 patients received azacytidine (Vidaza) in the dose of 75 mg/m<sup>2</sup> × 7 every 28 days and 4 patients were treated with decitabine (Dacogen) in the dose of 20 mg/m<sup>2</sup> × 5 every 28 days. Median number of cycles administered was 10.8 (range: 3–31). In the transplanted group, 8 patients were transplanted upfront and 10 patients were pretreated with combination chemotherapy, 8 patients received myeloablative conditioning and 8 patients were transplanted after reduced conditioning regimen. A hematologic response to HMA (CR, PR, hematologic improvement) was observed in 15 patients (61.5%), CR was achieved in 8 patients (31.8%). In SCT group, engraftment was achieved in 14 out of 16 patients, 8 patients died after SCT ( 5 on complications related to SCT, 3 patients relapsed). No difference in 1 year survival was observed between both the groups (65.6% for HMA vs. 62.5% for SCT), however, median overall survival (OS) was 19.0 months in HMA treated group compared to 47.6 months in SCT group (*P*=0.03). In a recent analysis performed at 48 months after starting the treatment, 2 patients treated with HMA (7.7%) and 6 transplanted patients (37.5%) were alive, 16 patients in HMA group and 3 patients in SCT group relapsed. Estimated 5 years

survival was 31.3% in SCT group and only 3.8% in HMA group (*P*=0.001). No significant differences in results and adverse effects of treatment were observed between patients aged 51–60 years and those older than 60 years in both HMA and SCT groups. Our results confirm previous observations showing that despite a promising effect of HMA resulting in hematologic response in more than 50% of elderly patients with advanced MDS, allogeneic SCT still represents the only potentially curative treatment connected with long-term survival in a significant number of patients even in this MDS patients subgroup.

**Disclosure of conflict of interest:** None.

**P663**

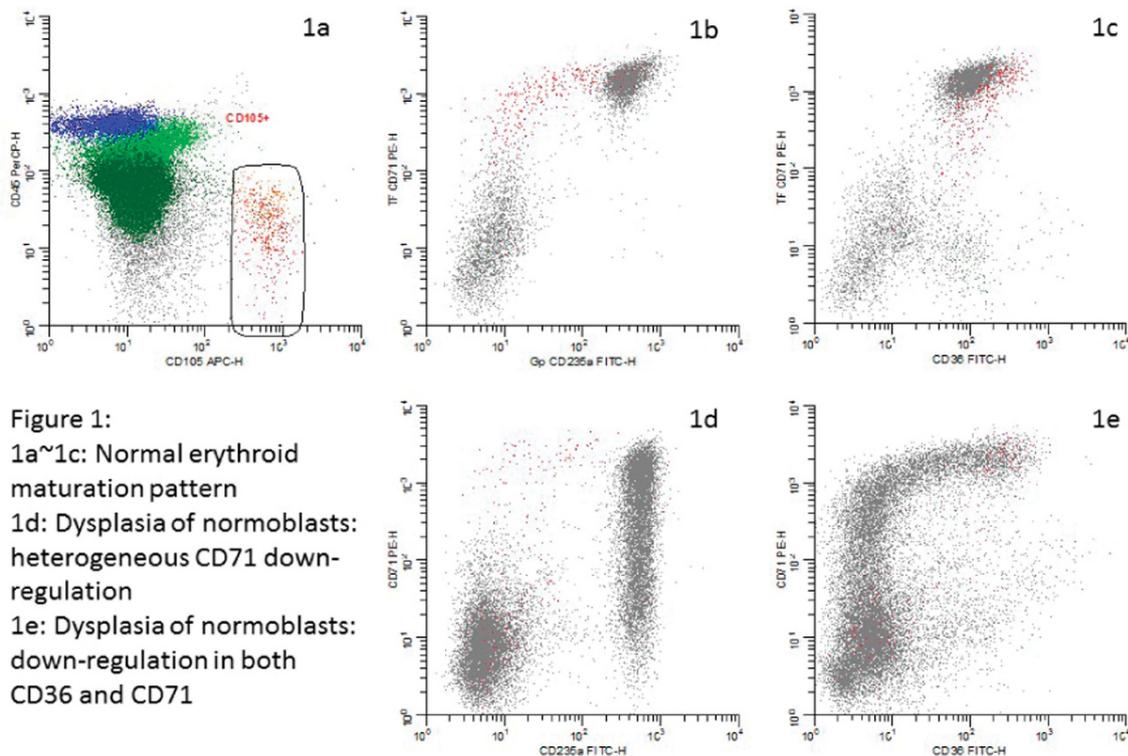
**Immunophenotypic assessment of erythroid dysplasia by a simplified cocktail in myelodysplastic syndromes in Taiwan**

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Abnormalities of erythroid lineage are frequently observed on morphology in myelodysplastic syndromes (MDS). However, their phenotypic abnormalities are difficult to be evaluated by conventional flow cytometry (FC). From October, 2015, to November, 2016, thirty-six consecutive bone marrow (BM) samples of morphologically confirmed MDS were evaluated for erythroid lineage by a cocktail of 2-tube, 4-color, flow cytometry: CD45-PerCP/CD105-APC/CD71-PE/CD235a-FITC and CD45-PerCP/CD105-APC/CD71-PE/CD36-FITC. Non-MDS control of twelve BM samples were also tested. Down- or up-regulation of one specific antigen is defined by more than 0.5 log deviation from normal erythroid maturation pathway. In the stage I and II erythroblasts (proerythroblasts and basophilic normoblasts), 50% of FC abnormalities (18/36 BM

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**Figure 1:**  
 1a~1c: Normal erythroid maturation pattern  
 1d: Dysplasia of normoblasts: heterogeneous CD71 down-regulation  
 1e: Dysplasia of normoblasts: down-regulation in both CD36 and CD71

samples) were found. Whereas, 94% of FC abnormalities (34/36 BM samples) were observed in down-stream maturing normoblasts, which included down-regulation in CD71 (62%), CD235 (24%), CD36 (59%), and aberrant up-regulation in CD36 (3%). There were two cases of MDS, EB-2, although erythroid aberrancy can not be found, FC did disclose significant aberrancy on myelomonocytic lineages. On the other hand, all the normal control BM samples revealed no any erythroid phenotypic abnormality. Our study suggests this simplified cocktail of 2-tube, 4-color, FC is very sensitive and useful in the assessment of erythroid phenotypic abnormalities in MDS, especially in the stages of maturing normoblasts.

**Disclosure of conflict of interest:** None.

**P664**

**Impact of T-cell depletion on outcome of patients undergoing allogeneic hematopoietic cell transplantation (HSCT) for myelodysplastic syndrome (MDS)**

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MDS is a clonal haematological disorder and HSCT is the only curative option. In the transplant unit of Geneva University Hospital, we use partially T-cell depleted graft (TDEP) to reduce graft-versus host disease (GvHD). Here, we compared 3-years overall survival (OS), progression free survival (PFS), GvHD-free/relapse-free survival (GRFS), relapse incidence (RI) and transplantation-related mortality (TRM) between TDEP patients and non TDEP ones allografted for MDS. We also evaluated the impact of TDEP on acute and chronic GvHD. We analyzed 62 consecutive patients (44% were female, median age of 48 (range: 18–70) allografted for MDS (median EBMT risk score of 3, median disease risk index of intermediate risk) over a 19-year period (1998–2016) with MAC conditioning for 66% and RIC for 34%. Median time from diagnosis to HSCT was 7.5 months (range: 3–86). PBSC (90%) or BM (10%) grafts were from identical siblings (45%), MUD (42%) or MMUD (13%). T-cell depletion was performed for 52% of patients. Median follow-up was 4.6 years (range: 0–15). There was no significant difference between TDEP and non-TDEP for patient characteristics. OS, PFS were estimated using the Kaplan–Meier method. Cumulative incidence estimates of TRM and GvHD were calculated with RI defined as competitive events by the Fine and Gray method. 3-years OS for all patients was 42 ± 14%, PFS 40 ± 14%, GRFS 26 ± 12%, RI 37 ± 13% and TRM 25 ± 12%. Three-years OS for TDEP patients and for non TDEP ones was not different (48 ± 18% and 34 ± 21% respectively, *P* value 0.317) (Graph). Similarly, there was not difference

between TDEP and non TDEP patients for 3-years PFS (48 ± 18% and 28 ± 20%, *P* value 0.321), 3-years GRFS (32 ± 17 vs 19 ± 17, *P* value 0.111) (Graph), 3-years RI (36 ± 18% and 37 ± 20%, *P* value 0.622) and 3-years TRM (26 ± 16% and 23 ± 18%, *P* value 0.933). Finally, TDEP had no significant impact on 3-years grade 2–4 aGVHD when compared to the non TDEP (26 ± 18% and 31 ± 16%, *P* value 0.656). It had not either on 3-years cGVHD (26 ± 18% and 28 ± 34%, *P* value 0.637). Our study shows that TDEP is feasible on patients undergoing HSCT for MDS and does not make the outcomes worse compared to non TDEP (OS, PFS, RI and TRM). Unexpectedly, TDEP does not significantly reduce the incidence of acute or chronic GVHD. However, the number of patients is small and the period span is long. These finding should be confirmed prospectively in larger cohort.

**Disclosure of conflict of interest:** None.

**P665**

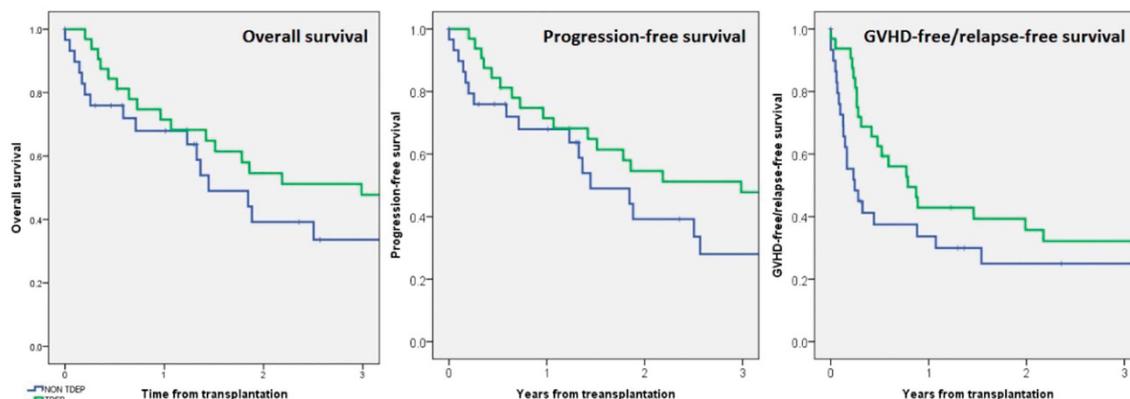
**Mutational pathway and dynamics may not be prognostic in patients with myelodysplastic syndrome receiving hypomethylating agent pre-treatment for allogeneic stem cell transplantation**

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Hypomethylating agent (HMA) is commonly used as a bridge therapy to prevent leukemic transformation prior to selection of a donor for allogeneic stem cell transplantation (SCT) in patients with myelodysplastic syndrome (MDS), and showed low toxicity. Although its roles are known, the underlying genetics and clonal dynamics upon HMA treatment has not been systematically examined using serial samples, especially in allogeneic stem cell transplantation (SCT) setting. In this study, we performed targeted serial sequencing on 66 bone marrow samples from 22 MDS patients treated with HMA for bridging of allogeneic SCT. To perform targeted deep sequencing, BM mononuclear cells before HMA treatment and, and fractionated T-cell samples (CD3+ fraction) as controls were taken before HMA treatment. Analysis of genetic mutations were performed using targeted resequencing by Illumina Hiseq 2000 (Sureselect custom probe set targeting

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entire exon regions of a myeloid panel consisting of 84 genes). All 22 patients received HMA (Decitabine: 15, azacitidine: 7), and the median number of cycles was four (range: 2–12). The overall response rate for HMA pre-treatment was 55%: there were four cases of complete remission (CR) (18%), six cases of marrow CR (27%), and two cases of hematologic improvement (9%). Targeted sequencing revealed 37 mutations in 16 patients (16/22, 73%) with median of 2 mutations per patient (range: 2–5). Mutated genes were then grouped into 8 biological pathways, defined in The Cancer Genome Atlas (TCGA) AML study. The most frequent biological pathway at diagnosis was DNA methylation (32%), followed by activated signaling (27%), chromatin modifiers (18%), tumor suppressors (18%), spliceosome (14%), cohesin complex (9%), *NPM1* (4%), and myeloid transcription factors (TFs) (4%). When assessing the difference in pattern of variant allele frequency (VAF), we found the significant reduction of VAFs in four (25%) patients after HMA. With a median follow-up of 63.4 months, 5-year overall survival (OS) were 69.6% (95% CI, 49.0–90.2). There was no significant difference in OS according to the presence of mutations in each biological mutational pathway (all,  $P > 0.05$ ). To identify prognostic value of mutational dynamics, we sub-classified the change of variant allele frequencies (VAFs) after median fourth cycles of HMA [no mutated or reduction of VAFs (11 patients) vs. stable or increased (11 patients)]. However, there was no significant association between the dynamic of mutation and OS ( $P = 0.374$ ). These data show that HMA using as bridge therapy for allogeneic SCT in MDS patients is insufficient to achieve the sufficient molecular responses and, mutational pathway and dynamics may not prognostic in this clinical setting. To clearly demonstrate the role of HMA pre-treatment in MDS, systematic assessment on a larger cohort is necessary.

**Disclosure of conflict of interest:** None.

## Solid tumours

### P666

#### Any role of high-dose chemotherapy in mediastinal non-seminoma germ cell tumors?

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Among germ cell tumors, primary mediastinal nonseminoma germ-cell tumors (PMNSGCTs) have the poorest outcome with 5-year overall survival ranging from 40 to 45%. Indeed, the presence of mediastinal location defines *per se* a “poor prognosis” category according to the IGCCCG classification. This clinically and biologically distinct disease entity is associated with lower complete response rates to chemotherapy (CT), high rates of relapse and disappointing results from salvage CT. Current standard first line treatment for patients with mediastinal primary location is still four cycles of PEB, as for all IGCCCG poor-prognosis patients. We have reviewed available data present in the literature, including recommendations and expert opinions, on the use of high-dose chemotherapy (HDC) with autologous stem cell support in PMNSGCTs. The use of HDC as both early intensification (that is, first-line setting) and at disease recurrence (salvage setting) have been reported in small cohorts of patients. According to the largest retrospective comparison, it has been suggested that HDC, given up-front, may produce a 15% to 20% absolute improvement in survival compared with standard dose CT. Studies of the EBMT suggest that responsive disease after induction therapy may have a better outcome. Mediastinal primary had salvage rates by HDCT of less than 15% based on an international multicenter analysis and an EBMT study. The use of HDCT in PMNSGCTs warrants further investigation,

preferably with the use of modern HDCT strategies (that is, multiple carboplatin and etoposide courses). While HDC cannot be routinely recommended in PMNSGCTs, selected patients with chemosensitive disease may benefit from early intensification. A retrospective analysis evaluating the large EBMT database is ongoing; results will be presented at the meeting.

**Disclosure of conflict of interest:** None.

### P667

#### High dose therapy and autologous stem cell transplantation in gynaecological malignancies: A monocentric retrospective study

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High-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) has been established as a treatment option in many relapsed hematologic malignancies. However, in spite of many small trials, there still is no proven role for this treatment in solid tumors including most gynaecological epithelial carcinomas. However, in some recurrent non-epithelial ovarian cancers, such as sex cord stromal tumors, germ cell tumors, neuroendocrine gynaecological tumors and gestational trophoblastic disease, some studies suggest a possible role for HDT followed by ASCT. We performed a monocentric retrospective descriptive analysis of all patients diagnosed with gynaecological malignancies and treated with HDCT followed by ASCT in our center. Clinical, laboratory, pathological and imaging data were collected and analysed, together with information on treatment and outcome. Eleven patients were included in this analysis, with a median age of 29 years (range: 14–56) at time of diagnosis. Eight patients suffered from ovarian neoplasia. At time of diagnoses 6 patients showed metastatic disease. First line therapy consisted of surgery ( $n = 4$ ), chemotherapy ( $n = 2$ ) or a combination of both ( $n = 5$ ). Median time to progression after first line therapy was 39.8 months (range: 0–192) with a median time between primary diagnosis and start HDT of 54.7 months (range: 4–306). Three patients underwent single ASCT, whereas the other 8 patients had a tandem ASCT, with a median time of 2 months between first and second HDT (range: 1–4). Treatment related toxicity was manageable, although there was 1 treatment-related death. At last follow up 5 patients (45%) were still alive with a median follow up of 3.9 years (range: 0.25–15.1) after last ASCT for all patients. Of the 6 deceased patients 5 died with progressive disease. Although the number of patients is very small, this retrospective study shows that HDT and ASCT is feasible in heavily pretreated patients with relapsed/refractory gynecological malignancies, although further studies are mandatory for optimal selection of patients, histological subtype and timing of HDT during the disease course.

**Disclosure of conflict of interest:** None.

### P668

#### The human endogenous retroviruses R, H, K and P (HERV-R, -H, -K, -P) are overexpressed in the tumor of colorectal cancer patients

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The Human Endogenous Retroviruses (HERVs) are remnants of ancient exogenous retroviral infections of the humans: they represent about 8% of the human genome<sup>1</sup>. The basic genes of HERVs are group-specific antigen (*gag*), polymerase (*pol*) and envelope (*env*); there are also two regulatory regions,

**Table 1: Expression Fold Difference of HERVs in different biological samples of patients**

	HERV-R	HERV-H	HERV-K	HERV-P
<b>PBMCs</b>	1	1	1	1
<b>NORMAL tissues</b>	1.35	1.08	1.11	3.2
<b>CANCER tissues</b>	0.001	24.45	10.03	78.37

Long Terminal Repeat (LTR), located at 5' and 3' ends. Several reports have shown that HERVs may play a role in the development of autoimmune diseases, such as multiple sclerosis<sup>2</sup>. Additionally the existence of a strong relationship between HERVs expression and cancer, based on the mRNA expression profile of HERVs in normal and cancer tissues has been suggested<sup>2</sup>. The increased level of expression level of HERV-H in colorectal cancer (CRC), a major cause of cancer death worldwide has been already shown. The aim of the study was to analyse the expressions of *env* genes of HERV-R, HERV-H, HERV-K and HERV-P in the peripheral blood mononuclear cells (PBMCs), in the tumor and in the adjacent normal tissues of 20 colorectal cancer patients. A group of control composed by PBMCs from 46 healthy subjects was also included. RNA was isolated from the biological samples and a reverse transcription assay was conducted. Quantitative Real Time PCR was performed to evaluate the expression of the HERVs *env* gene. All the *env* genes were related to the expression of an housekeeping gene, GAPDH. The quantification was carried out using comparative Ct method and the difference between the levels of *env* gene expression in PBMCs, cancer and adjacent normal tissue was given by Fold Difference. Fold difference values were relative to a calibrator: first the PBMCs of patients and then PBMCs of control healthy group.  $\Delta$ Ct values were analysed using the paired sample T-Test, followed by a Bonferroni correction. Higher levels of expression of HERV-H, HERV-K and in particular HERV-P were found in tumor tissues, as compared to PBMCs and to adjacent normal tissues of patients, with an increase of 24-, 10- and 78-folds, respectively. The  $\Delta$ Ct distribution of HERV-H, HERV-K and HERV-P in cancer tissues were statistically significant ( $P < 0.05$ ) (Table 1).

The expression of HERV-H, HERV-K and HERV-P *env* gene resulted increased in the colorectal tumor tissues also when compared with the PBMCs of the healthy controls (5-, 15- and 26-folds, respectively). The  $\Delta$ Ct distribution of HERV-H, HERV-K and HERV-P in tumor tissues were statistically significant ( $p < 0.05$ ). No difference of expression was observed between PBMCs of healthy controls, PBMCs and normal adjacent tissues of patients (Figure 1). HERVs *env* gene expression cannot be used as a diagnostic biomarker, but it is conceivable that HERVs are directly involved in the pathogenic process of cell transformation and, if the protein expression will be demonstrated, the protein of HERVs *env* gene could be the target for new immunotherapy strategies against colorectal cancer.

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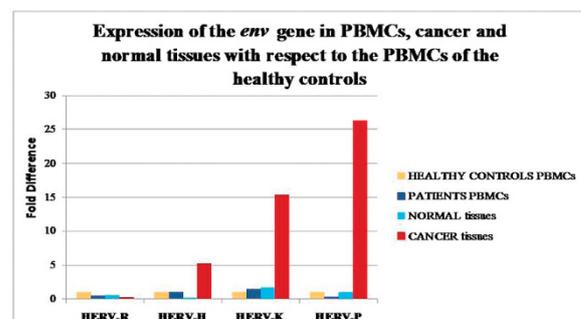


Figure 1: Comparison between the expression of the *env* gene in PBMCs, cancer and normal adjacent tissues with respect to the PBMCs of the healthy controls

**Disclosure of conflict of interest:** None.

## General

### P669

#### **A Biosimilar G-CSF filgrastim is as effective as a reference drug however it is not as cost effective as it supposed to be and by the way no impact on the health care system**

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Biosimilars are up to 1000 times the size of small molecule generic drugs and far more structurally complex. Additionally biosimilars are manufactured in living cell lines using processes that cannot be exactly replicated from one manufacturer to the next. A biosimilar cannot be identical to its reference biologic drug. With 67 billion dollars in global sales of biologic medicines anticipated to go off patent by 2020. This leads to fast production of biosimilar drugs. Besides, it is expected that biosimilar drugs will be more cost effective than the reference drugs and will have a meaningful impact on health care systems around the world. Aim: To compare biosimilar filgrastim (Leucostim) with two reference G-CSF filgrastim (Neupogen) and lenograstim (Granocyte) in the context of safety, efficacy and cost effectiveness. Records of patients with multiple myeloma (MM) whom underwent autologous stem cell transplantation (ASCT) and received G-CSF 5 microgram/kg/day from +day 5 until engraftment were

retrospectively evaluated 60 MM patients were treated with high dose melphalan and ASCT at the Ankara University School of Medicine Bone Marrow Transplantation Unit between 2013 and 2016. The median age was 59 (38–75 years) with 55% male. Patients were divided into three groups ( $n=20$ ) whom received reference filgrastim (Neupogen), lenograstim (Granocyte) and biosimilar filgrastim (Leucostim): groups A, B and C respectively. The total cost of each G-CSF in dollars was calculated by one package of G-CSF multiplied by total used days. Chi-square, Mann-Whitney U and Kruskal-Wallis tests were used for analyses of variance. The percentage of patients who received melphalan 200 mg/m<sup>2</sup> were 80, 85, 80 in groups A, B, C respectively ( $P=0.9$ ). There was no statistically significant difference between the engraftment day of neutrophil 500 and 1000; platelet 20 000 and 50 000 in the groups. ( $P=0.07$ ,  $P=0.55$ ,  $P=0.33$ ,  $P=0.81$  respectively) The median numbers of G-CSF administered days were 7(5–18), 8 (5–12), 7(4–16) in groups A, B, C, respectively. Even though there was no statistical difference between the numbers of days ( $P=0.23$ ), the total cost in dollars was statistically difference between A vs B and C vs B (both  $P<0.0001$ ) and there was no statistical difference between A vs C ( $P=0.89$ ), total cost in dollars as follows: 155\$(112–288\$), 416\$(260–624\$) and 166\$(81–250\$) for the group A, B and C respectively. Our results demonstrate that biosimilar G-CSF Leucostim is highly similar to existing licensed biologic products in Turkey with no clinically meaningful difference in terms of safety and efficacy. On the other hand it as a biosimilar does not have a meaningful impact on the cost savings to the health care system as expected when compared with reference filgrastim.

**Disclosure of conflict of interest:** None.

#### P670

##### **A new mechanism to control ABL tyrosine kinase activity in STIs (Signal transduction inhibitor) treated CML patients via PRX II (Peroxiredoxin II)**

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In this study, we investigated the roles of PRX II, one of 3 critical peroxidases besides catalase and GPXs, in CML primary cells at diagnosis and remission while patients were treated with STI (Signal Transduction Inhibitor) and tested the same roles in Imatinib(IM) sensitive Ph+ cell lines and resistant cell lines as well. Newly diagnosed CML cells, IM resistant K562 cells and parental K562 cells were treated STIs and analyzed western blot assay to detect BCR-ABL, phosphorylated BCR-ABL and PRX2 protein expression level. We added N-Acetylcysteine (0–5mM, 6hr) to K562 cells to show antioxidant effect of imatinib and analyzed DCF-DA detection for intracellular ROS level and western blot for PRX2 protein level. MTT assay was performed to detect cell death by NAC time-dependent treatment of 5mM NAC(0, 24, 40, 48hr). Imatinib resistant K562 cells were established by treatment of gradual increment of imatinib. We also repeatedly investigated the effects of IM therapy using PRXII over-expressed K562 cells by transfection. At diagnosis of CML, ROS level was elevated and PRX II was either absent or significantly suppressed. As Ph chromosomes were decreased with STIs, suppressed or absent PRXs levels were restored to the level of normal individuals. These findings were also inversely correlated with the level of Ph chromosomes in the cases of disease progression and re-remission with further treatment. When STI were treated in Ph positive cell line, we found decreased cell survival and ROS level by MTT assay and DCF-DA methods respectively, but elevated PRX II by western blot. By the treatment of NAC into Ph+ cell lines, the level of DCF-DA was decreased and MTT level was down, but PRX II level was elevated. Interestingly, the level of BCR-ABL oncogene were

decreased in PRX II transfected cells. Meanwhile, we observed that PRX II restoration was mild or weak in Imatinib resistant K-562, which we established in our lab. The importance of the roles of ROS and its PRX II, antioxidant enzymes in CML is further established by our work. Our finding may contribute to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-BCR kinase to the substrate resulting apoptosis of Ph+ cells. Furthermore, our finding may be useful to overcome the STIs resistant CML in the clinics in the future.

**Disclosure of conflict of interest:** None.

#### P671

##### **Allogeneic hematopoietic stem cell transplantation for the treatment of mucopolysaccharidosis**

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Mucopolysaccharidosis (MPS) is a lysosomal storage disorder caused by deficient activity of the iduronate-sulfatase. This leads to accumulation of glycosaminoglycans (GAGs) in the lysosomes of various cells, which causes progressive multi-system involvement with ensuing death. The aim of this study was to exploit the effect of treatment with allogeneic hematopoietic stem cell transplantation and administration of high doses of cyclophosphamide early after haploBMT in these cases. We retrospectively reviewed data from 3 MPS patients (2 cases MPS II, and 1 case MPS I). The two MPS II patients were 44-month-old and 35-month-old boy and the MPS I patient is a 84-month-old girl at the time of transplantation. The reduced-intensity of Bu+Flu conditioning regimen in allo-HSCT for these patients was as follows: busulfan 4 mg/kg at 5–2 days before transplantation, fludarabine 40 mg/m<sup>2</sup> at 6–3 days before transplantation. Graft-versus-host disease (GVHD) prophylaxis: rabbit antithymocyte globulin 2.5 mg/kg daily at 5–3 days before transplantation, short-course methotrexate, posttransplantation high-dose cyclophosphamide on days +3 and +4 was followed by mycophenolate mofetil and cyclosporine. The donors all were their HLA-haploidentical father. These three patients' Neutrophil engraftment occurred on +14d, +12d and +15d after transplantation respectively, platelet engraftment occurred on day +14d, +10d and +15d after transplantation respectively. Complete donor type engraftment was confirmed by Short Tandem Repeat-Polymerase Chain Reaction (STR-PCR) on day 14 after transplantation. No regimen-related toxicity occurred, GVHD and graft failure were not observed. 1 month after transplantation, the activity of the iduronate-sulfatase was increased to normal. The motion of metacarpophalangeal joints ameliorated, regression of hepatosplenomegaly, the neurocognitive function improved. Allogeneic hematopoietic stem cell transplantation is an effective measure to treat patient with MPS at least MPS II and MPS I. The reduced-intensity conditioning regimen was helpful to decrease the regimen-related toxicity. Post-transplant cyclophosphamide approach successfully used and reduced the incidence of GVHD.

**Keywords:** Mucopolysaccharidosis, Allogeneic stem cell transplantation, Posttransplant cyclophosphamide.

**Disclosure of conflict of interest:** None.

#### P672

##### **Alternative donor hematopoietic stem cell transplantation using busulfan, fludarabine, and thymoglobulin conditioning for patients with chronic granulomatous disease**

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This study aimed to evaluate the feasibility of alternative donor hematopoietic stem cell transplantation (HSCT) using busulfan, fludarabine, and thymoglobulin conditioning for patients with chronic granulomatous disease (CGD) who lack an HLA-matched familial donor. Medical records of 11 consecutive patients who received alternative donor HSCT between May 2010 and May 2016 were reviewed, and the transplant-related outcome measures were analyzed retrospectively. The donor source was unrelated peripheral blood (PB) in 4, unrelated cord blood (CB) in 4, and haploidentical father in 3 patients. Only 2 transplants (8/8 allele-matched unrelated PB) were HLA-matched according to current standards relevant to the donor type. The conditioning regimen was uniform; fludarabine 40 mg/m<sup>2</sup> on days -8 to -4, busulfan 3.2 mg/kg/d (or 120 mg/m<sup>2</sup>/d) on days -6 to -3, and thymoglobulin 2.5 mg/kg/d on days -3 to -1 (or on days -8 to -6 in CB recipients). All but one patient were male and their median age at transplantation was 6.5 y (range: 1.1–26.3). One patient who received a cord blood graft suffered from primary engraftment failure, while the other 10 patients were successfully engrafted with their chimerism levels ranging from 66% to 100% (median 100%) at 1 month post-transplant. The median days to neutrophil and platelet engraftment were 12.5 (range: 11–22) and 27 (range: 11–47), respectively. Among the 10 patients engrafted, one patient experienced secondary graft failure which was rescued by a second transplantation. The remaining one patient who failed to engraft was also rescued with a haploidentical graft from his mother. Eight patients (73%) developed CMV antigenemia, and one of those patients developed CMV hepatitis. Three patients developed grade 3 acute GVHD which were manageable. One patient who developed grade 4 hepatic GVHD eventually died. Two patients developed extensive chronic GVHD, but became free of immunosuppressants after a complete resolution in one and with remaining stable mild joint contractures in the other. Including 2 patients who were rescued by additional transplantation, 10 patients are alive with their latest chimerism levels ranging from 86.8% to 100% (median 100%). The estimated 5-y overall survival rate was 85.7% with a median follow-up of 49 months (range: 6–72). Even though the majority of our cohort underwent a mismatched transplantation, the survival rate was excellent. While conditioning with busulfan, fludarabine, and thymoglobulin seems feasible for alternative donor HSCT in patients with CGD, special attention needs to be paid on CMV infection and severe GVHD which might offset the high survival rate.

**Disclosure of conflict of interest:** None.

#### P673

##### **Amebiasis in hematopoietic stem cell transplantation: An endemic region analysis**

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Diarrhea is a common infectious complication in patients who had hematopoietic stem cell transplantation<sup>2</sup> so, we aimed to detect *Entamoeba histolytica* ratio before engraftment, among 375 patients who had diarrhea after periferic hematopoietic stem cell transplantation (PHSCT) in our clinic. Allogenic PHSCT patients had a median age of 29 (range: 15–63) and autolog PHSCT patients had a median age of 54 (range: 18–74). Diarrhea is described as an abnormal increase in the frequency (3 or more times per day), volume or liquidity of stools. We based upon this description in this study. We made stool examination in the first day of diarrhea. As stool examination, we used direct microscopic evaluation and adhesin antigen test specific for *E.histolytica* with Enzyme Linked Immunosorbent Assay (ELISA), *E. histolytica* II, Techlab, Blacksburg, USA). We accepted *E.histolytica* positivity as

detecting cyst or/and trophozoite in stool and antigen test positivity at the same time. In our study, 185 of 375 patients had diarrhea in the first 28 days of PHSCT. Diarrhea was found in 139 of 242 in autologous PHSCT patients (57%), 21 of 63 patients in allogenic PHSCT with non- myeloablative conditioning regimen (33%) and 25 of 70 patients in allo PHSCT with myeloablative conditioning regimen (36%). Diarrhea occurred at +8th day of transplantation and the median duration of diarrhea was 3 days. *E. histolytica* positivity was found 46 of 185 patients (25%) who underwent PHSCT within first 28 days of transplantation. Infection is an important mortality and morbidity factor for patients who had hematopoietic stem cell transplantation, when especially before engraftment (between 0–30 days).<sup>1</sup> Autologous PHSCT patients were elderly, with poor self-care and low socioeconomic status individuals. *E. histolytica* is a frequent pathogen in post-transplant diarrhea at endemic regions. Prophylactic metronidazole treatment should be used routinely for autologous PHSCT as in allogenic PHSCT.<sup>3</sup> Patients and companions should be tested for *E.histolytica* before autologous/allogenic PHSCT in endemic regions. Prophylactic treatment for amebiasis and scanning patient/companions could be a part of solution for post PHSCT diarrhea.

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#### P674

##### **Autologous haematopoietic stem cell transplant in multiple sclerosis: Updated results from the Pan-London MS group**

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Despite the emergence of disease modifying therapies (DMTs) for multiple sclerosis (MS) a cohort of patients with aggressive disease have ongoing progression/relapse, associated with progressive disability. Autologous Haematopoietic Stem Cell Transplantation (AHSCT) has been used worldwide for aggressive MS with inflammatory changes on MRI. We update on a UK single centre experience of AHSCT in MS. A retrospective audit of AHSCT performed for MS from 2012 to 2016 at 1 UK centre (King's College Hospital) was undertaken. Patients were selected for transplantation based on persistent clinical relapses (relapsing-remitting MS) or secondary progressive neurological disability with MRI lesion activity despite use of at least 1 DMT. Primary progressive patients were also eligible if new/active MRI lesions were demonstrable. Follow-up included clinical evaluation, EDSS assessment and MRI scanning. We report our preliminary findings. As of November 2016, 30 patients (16 female, 14 male, 18 RRMS, 10 SPMS, 2 PPMS) had received AHSCT. Mean age at transplant was 40.6 years (range: 22–57). The mean baseline EDSS was 5.3 (range: 2.5–8.0). 29 patients underwent cyclophosphamide/ATG conditioning, while 1 received BEAM/ATG. Whilst conditioning and stem cell infusion were well tolerated there was a high rate of infections, with 23/30 patients developing a culture confirmed infection. Reactivation of EBV and CMV were observed in a number of patients (21 and 8, respectively) while a number of delayed herpes zoster infections were also seen (4 cases of shingles and 2 of disseminated varicella infection in patients who had previously experienced it in childhood). Median follow-up was for 361 days (63–1479). Of patients with a

formal 6 month assessment ( $n = 16$ ), 4 had a stable EDSS, 6 had an improved score (median improvement 0.5, range: 0.5–2.5) and 6 had a deterioration in their score (median 0.5, range: 0.5–1.0). At 12 months ( $n = 11$ ), 1 had a stable EDSS, 4 had an improved score (median 0.75, range: 0.5–1) and 6 had a deterioration in score (median 0.5, range: 0.5–1.5). At 24 months, two patients assessed both had improvements in EDSS scores (median 1, range: 0.5–1.5). For patients who underwent MRI at 6 month follow-up ( $n = 14$ ), 10 had a stable lesion load, 2 demonstrated improvement in lesions and 1 had a new lesion (the remaining MRI was difficult to read due to a high baseline lesion load in this patient). 4 Patients had MRI's at 12 months; 3 were stable and 1 demonstrated a reduction in lesion load. To date, no patients have developed secondary malignancies or autoimmune diseases. Of patients with follow-up data, 4/18 RRMS patients experienced suspected clinical relapses following HSCT—only one had a new lesion on MRI (with no gadolinium enhancement). 3 of the 4 received steroids to treat these relapses (it is unclear if the remaining patient received treatment). 1 patient tried a new disease modifying therapy (1 dose of rituximab) following HSCT. AHSCT in this cohort was feasible with universal mobilisation and harvest. Whilst conditioning and stem cell infusion were well tolerated, there was a high rate of infectious complications in the neutropenic phase. However, the transplant related mortality was 0% despite significant levels of disability amongst this patient cohort. AHSCT remains a treatment option to be further investigated in this difficult cohort of patients.

**Disclosure of conflict of interest:** None.

#### P675

##### **Baseline peripheral blood CD34+ cell count correlates with the effectiveness of allogeneic stem cell mobilization in healthy donors after administration of G-CSF**

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Peripheral blood (PB) Stem Cells (SCs) mobilized with G-CSF are the first-choice source for allogeneic transplantation. We carried out a prospective study on healthy donors (HDs), to identify donor characteristics that could influence the effectiveness of mobilization with special focus on the value of the basal CD34+ cell count. Sibling HDs were analyzed in a prospective study. We tested somatic variables (sex, age, weight, height, volemia) and, basal blood counts (White Blood Cell, Peripheral Blood Mononuclear Cell, Platelets, Hematocrit, Hemoglobin, CD34+ cell). HDs received G-CSF subcutaneously at a dose of 10 µg/kg day. Two different determinations of CD34+ cells were done in each donor: baseline (before G-CSF administration) and in PB on the morning of the fifth day (after G-CSF administration). 128 consecutive HDs (65 males) with a median age of 43 years were enrolled. The mean value of CD34+ on day 5 was 90.8 cells/µL, while the median value was 75.5 cells/µL. We performed two multivariate analyses either by using median regression (to predict the median value of CD34+ on day 5) according to the values of CD34+ at baseline, the first adjusted by gender, age and blood volume and the second by gender, age and BMI. Results of both models indicate that from basal CD34+ values  $< = 1$  to values ranging between 3 and 4 cells/µL, predicted median values of CD34+ on day 5 significantly increase, from 54.6 to 92.8 cells/µL for model adjusted by blood volume, and from 49.9 to 92 cells/µL for model adjusted by BMI. Baseline, PB CD34+ cell count correlated with the effectiveness of allogeneic PBSCs mobilization and could be useful to plan the collection.

**Disclosure of conflict of interest:** None.

#### P676

##### **Clinical analysis of autologous peripheral blood hematopoietic stem cell mobilization regimen in 56 multiple myeloma patients**

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Comparison of efficacy between chemotherapy plus granulocyte colony stimulating factor (G-CSF) and chemotherapy plus G-CSF and granulocyte-macrophage colony stimulating factor (GM-CSF) for mobilization of peripheral blood stem cells (PBSC) and hematological recovery post-transplantation in patients with multiple myeloma (MM). A retrospective study of autologous peripheral blood stem cell (APBSC) mobilization data of 56 MM patients who treated with chemotherapy plus G-CSF or chemotherapy plus G-CSF and GM-CSF from May 2008 to July 2016. The mobilization efficacy and hematopoietic recovery were analyzed. A total of 65 stem cell mobilizations were performed in 56 MM patients. In the univariate analysis, successful collection rate of single harvest in female and in patients at ISS stage III, R-ISS II/III and chemotherapy plus G-CSF was lower ( $P < 0.05$ ). However, age ( $\leq 60$  yrs vs  $> 60$  yrs), subtype, D-S staging (I+II vs III), cycles of chemotherapy before mobilization ( $\leq 6$  cycles vs  $> 6$  cycles), disease phase before mobilization (PR vs CR) and interval diagnosis-mobilization ( $\leq 18$  months vs  $> 18$  months) were not correlated with the CD34+ cell collection and successful mobilization rate ( $P > 0.05$ ). In the multivariate model, rate of successful mobilization in patients who received chemotherapy plus G-CSF+GM-CSF mobilization regimen was high (OR = 12.009, 95%CI 1.961–73.537). The effect of mobilization regimen remained significantly ( $P = 0.007$ ). All patients successfully underwent hematopoietic reconstruction without transplantation-related mortality. Chemotherapy plus G-CSF +GM-CSF mobilization regimen can significantly increase the effect of APBSC mobilization and ensure the reconstruction of hematopoietic function after transplantation. This mobilization regimen is a safe and effective method of mobilizing APBSC.

**Disclosure of conflict of interest:** None.

#### P677

##### **Clinical efficacy of BK virus specific T-cells in treatment of severe refractory hemorrhagic cystitis after HLA haploidentical transplantation**

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Hemorrhagic cystitis caused by BK virus (BKV) is a significant complication of allogeneic hematopoietic cell transplantation (HCT). It is particularly common in the setting of HLA haploidentical transplantation and can be challenging to manage. Here we present a post haploidentical HCT patient who developed severe BKV haemorrhagic cystitis resistant to standard therapy and who responded to adoptive transfer of donor T cells enriched with anti-BKV specific cells. A 40 year old man underwent HCT for acute myeloid leukaemia with inversion of chromosome 3 and monosomy of chromosome 7 while in first complete remission. As he had no related or unrelated HLA identical donor, cells from his HLA haploidentical sister were used. On day +32 of this procedure he developed haemorrhagic cystitis. Supportive treatment was initiated and cystoscopy showed diffuse bleeding from his urinary bladder with blood clots. Urine PCR for BKV showed 5.2 billion copies/mL. Despite bladder irrigation, local therapy to

bladder mucosa and intravenous hydration, he failed to improve, so treatment with weekly intravenous cidofovir was initiated on day +38. His symptoms improved, but on day +72 he again deteriorated on weekly infusions of cidofovir. His immunosuppression was slowly tapered off without any graft versus host disease (GvHD) but without any significant effect on his hemorrhagic cystitis. He underwent bladder diathermy, was treated with intravesicular hyaluronate and with intravenous cidofovir, but continued to have frank haematuria with blood clots and significant lower abdominal pain. Although there was no evidence of obstruction his renal function deteriorated on cidofovir therapy. Hence we elected to trial adoptive anti BKV therapy. A leukoapheretic lymphocyte collection was used to prepare an anti-BKV T cell enriched product using the Clinimacs Prodigy and the cytokine capture system from Miltenyi Biotec. The eluted product contained 50% and 5% of CD4+ and CD8+ lymphocytes expressing IFN $\gamma$ + respectively and the CD4+/CD8+ dose adoptively transferred on day +86 of transplantation was  $0.34 \times 10^4$ /Kg. *In vivo* expansion of anti-BKV T cells in the patient was analysed weekly for the first month using the research grade peptivators BKV LT and BKV VP1 and the rapid cytokine inspector (CD4/CD8 T cell) kit. BK viral load was monitored by PCR in urine samples twice weekly. IFN $\gamma$ + anti-BKV reactive T cells were undetectable in the patient for the first two weeks after adoptively transfer of donor T cells. Twenty days after the adoptive transfer an increase in the CD4+ IFN $\gamma$ + population was observed, in response to the BKV VP1 peptivator. This observation correlated in time with a substantial decrease of the urine BKV viremia from 3.3 million copies/mL to 1360 copies/mL and a complete resolution of patient's symptoms. No GvHD, recurrence of urinary symptoms or any other problems have been observed to date (day +260 of transplantation, +174 days after the adoptive transfer). We are not aware of any other reports of successful adoptive anti BKV cellular therapy. Our experience suggests safety and efficiency of the use of anti-BKV T cell enriched products using the Clinimacs Prodigy and the IFN $\gamma$  capture system in HLA haploidentical HCT where BKV cystitis constitutes a significant complication. This opens the possibility of further clinical trials.

**Disclosure of conflict of interest:** None.

#### P678

##### **Comparable outcomes of haploidentical hematopoietic stem cell transplantation with post-transplantation cyclophosphamide and unrelated donor transplantation**

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Haploidentical donor (HD) has been used as an alternative stem cell source when patients do not have a HLA-matched related or unrelated donor. To overcome the HLA barrier, haploidentical stem cell transplantation (haploSCT) using post-transplantation cyclophosphamide (PTCy) has been conducted. Here, we compared the clinical outcomes of haploSCT using PTCy with those of unrelated donor transplantation. Eighty-two patients (28 from HD and 54 from unrelated donor [UD]) who underwent allogeneic hematopoietic stem cell transplantation (HSCT) in Seoul National University Children's Hospital from January 2013 to June 2016, were analyzed. There were no significant differences between HD and UD patients with respect to median age of patients, sex distribution, and diagnosis (HD: 17 acute leukemia [60.7%], 4 other malignancies [14.3%], and 7 non-malignant diseases [25.0%]; UD: 28 acute leukemia [51.9%], 3 other malignancies [5.6%], and 23 non-malignant diseases [42.6%],  $P=0.081$ ). The conditioning regimen of haploSCT included targeted busulfan, fludarabine and cyclophosphamide using PTCy, tacrolimus and mycophenolate mofetil for graft-versus-host disease (GVHD) prophylaxis. All patients showed engraftment except for a patient who underwent unrelated HSCT. Neutrophil engraftment of UD was faster than HD (median 11 days versus 15.5 days, respectively,  $P < 0.001$ ). However, there was no significant difference of platelet engraftment. Incidences of complications, such as hepatic venoocclusive disease, CMV

infection, and hepatic dysfunction, between both groups, were comparable, except hemorrhagic cystitis (HD: 32.1%, UD: 7.4%,  $P=0.004$ ). Moreover, cumulative incidence of acute GVHD (HD: 32.3%, UD: 44.7%,  $P=0.260$ ), severe chronic GVHD (HD: 8.4%, UD: 26.7%,  $P=0.059$ ), relapse (HD: 28.6%, UD: 25.1%,  $7P=0.323$ ) and non-relapse mortality (HD: 0%, UD: 9.7%,  $P=0.151$ ) were not significantly different. The overall and event-free survival of HD and UD were 85.4% vs 86.2% ( $P=0.703$ ) and 75.0% vs 75.9% ( $P=0.509$ ), respectively. The clinical outcomes of haploSCT using PTCy were comparable with those of UD, and a trend of lower cumulative incidence of severe chronic GVHD and non-relapse mortality was encouraging. It could be a promising alternative therapeutic option in pediatric HSCT.

**Disclosure of conflict of interest:** None.

#### P679

##### **Cryopreservation of peripheral blood stem cells for autologous transplantation in Republic of Macedonia**

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Cryopreservation of peripheral blood stem cells (PBSC) is an essential procedure during the process of autologous hematopoietic stem cell transplantation (ASCT) in the treatment of patients with hematological malignancies. The aim of this study is to present the 16 years experience with cryopreservation in the only single center for cryopreservation and stem cell transplantation in Republic of Macedonia. Autologous peripheral blood stem cells (PBSC) were cryopreserved from 295 patients treated with ASCT at University Clinic of hematology, Skopje, Macedonia. Statistical comparison of the safety of cryopreserved grafts of PBSC was performed with fresh bone marrow (BM) grafts as a historical control group of patients treated with ASCT. During 16 years of experience with cryopreservation of stem cells a total of 510 procedures were performed. The longest storage of cryopreserved PBSC was 7 years. We had 2 contaminated grafts with malignant cells and 9 unadministered grafts that are still stored under  $-198^{\circ}\text{C}$ . Median graft volume was 149.62ml (range: 60–200), median number of cryobags 4.4 (range: 2–8), median days in liquid nitrogen 33.89 (range: 2–330), median number of CD34+ cells was  $2.34 \times 10^6$ /kg (range: 0.1–5.7), median number of apheresis procedures was 2.15 (1–6), median amount of DMSO infused 20 ml (7–60). Time to engraftment was median 11 days (10–22). Statistical comparison between cryopreserved PBSC grafts and BM showed benefit for PBSC in the terms ( $P < 0.01$ ) of faster engraftment, less infective complications, less transfusion support and less hospital stay. In 253 patients (86%) DMSO related events were not registered during graft administration. In 41 patients (14%) mild to moderate DMSO related events were registered, as nausea in 34 patients (83.3%), vomitus in 20 patients (50%), tachycardia in 8 (20.8%), hematuria in 6 patients (16%) and 2 patients (4.16%) with bradycardia, hypotension, fever and high temperature during graft infusion. Cryopreservation of stem cells is a feasible procedure at our institution. There are some issues that have to be improved. The process is standardized with achieved engraftment in all transplanted patients.

**Disclosure of conflict of interest:** None.

#### P680

##### **Effectivity of a fludarabine based conditioning regimen in allogeneic hematopoietic stem cell transplantation for patients with severe aplastic anemia and over twenty years old**

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Severe aplastic anemia (SAA) is an anemia with bone marrow hypocellularity and caused by hematopoietic stem cell failure

(1). Allogeneic periferic hematopoietic stem cell transplantation (APHSCT) is a curative treatment choice (2). Although cyclophosphamide (CyC) and Anti thymocyte globulin(ATG) is accepted as standart conditioning regimen, especially for patients with high rejection risk, using fludarabine (Fu) based regimens show increased successful engraftment ratio with minimal toxic side effects (3). To the study, 20 SAA patiens who were transplanted from HLA matched sibling donors between the years 2010–2015 were included. The patients comprised of 13 male (%65) and 7 female (%35). Median age was 22 (range: 20–42). The median time from diagnosis to transplantation was 3 (range: 2–108) months. Conditioning regimen consisted of CyC (1200 mg/m<sup>2</sup>), Fu (120 mg/m<sup>2</sup>), ATG (Fresenius rabbit, 15 mg/kg). The median dose of stem cells was 7 × 10<sup>6</sup> stem cell/kg (range: 5–12). Methotrexate (10 mg/m<sup>2</sup> given four days) and cyclosporine (CycA) (3–5 mg/kg given 18 months) were applied for Graft versus host disease (GVHD) prophylaxis. All 20 patients ECOG performance status were good (0–1). Prior to transplantation only one of the patients received ATG-CsA, the others received only supportive treatment. After AHSCT, neutrophil engraftment was occurred at a median of 16 days (range: 11–20) and thrombocyte engraftment was occurred at a median of 14 days (range: 11–20). Posttransplant graft failure was observed in only one patient at tenth month and this patient had AHSCT again from the same donor with the same conditioning regimen. Acute GVHD didnot occur in any patient. The 5 (%25) of patients had common chronic skin/oral mucosa GVHD. These 5 patients received methylprednisolone (MP) and/or mycophenolate mofetil (MMF) in addition to the cyclosporine treatment. Extracorporeal photopheresis was applied to the two patients with chronic GVHD. All chronic GVHD patients had complete response to the immunosuppressive treatment with a median follow up time 46 months (range: 1–64). One patient died from sepsis. At 5 year overall-survival rate was 90%. Fu based conditioning regimen in AHSCT with young SAA patients has favorable results. Fu based regimen might be a gold-standard treatment in the future.

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#### P681

##### **Efficacy of hypomethylating agents administration before allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia, myelodysplastic syndrome, juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia**

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The aim of this study was to assess median overall survival (OS), median progression-free survival (PFS) and median event-free survival (EFS) in patients (pts) with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia (CMML), treated with hypomethylating agents (HMA) before allogeneic hematopoietic stem cell

transplantation (allo-HSCT). We analyzed data of 54 pts with AML (*n* = 15, 28%), MDS (*n* = 33, 61%), JMML (*n* = 4, 7%), CMML (*n* = 2, 4%). The median age was 33 (range: 0.3–61) years, the majority of patients were male (*n* = 29, 54%). According to the cytogenetic status, 22 (41%) of pts have been classified in the good, 13 (24%)–intermediate, and 18 (35%) in the poor risk group. Thirty two (60%) pts received 5-azacytidine (5-aza), 19 (35%) pts - decitabine (dec) and 3 (5%) pts - both drugs before allo-HSCT. Median number of HMA therapy cycles was 4 (range: 1–12). All pts received allo-HSCT. Allo-HSCT were performed from HLA-identical related (*n* = 11, 20%), HLA-identical unrelated (*n* = 30, 55%), HLA-mismatched unrelated (*n* = 5, 10%), and haploidentical (*n* = 8, 15%) donors. Response was achieved in 30% (*n* = 16) of pts after 1–5 (median 3) courses of HMA therapy: complete remission (CR) in 2 (4%), partial remission (PR) in 14(26%) of pts. Stabilization (S) was documented in 30 (56%) pts, in 8 (14%) pts there was disease progression (P) after beginning of HMA therapy. Median OS was 776 days (95% CI, 346–1205), and it was significantly better in pts with MDS (1036 days; 95% CI, 619–1452), than in AML pts (382 days; 95% CI, 134–1092) (*P* = 0.05). Median EFS was 661 days (CI 95%, 1–1346), it was significantly higher for those pts, who achieved CR, PR, S (965 days; CI 95%, 689–1224) and who was still in CR, PR, S at the moment of allo-HSCT (1062 days; CI 95%, 790–1333) in comparison with pts, who progressed during the HMA therapy (300days; CI 95%, 108–491, or being in P at the time of allo-HSCT (306 days; CI 95%, 171–442) (*P* = 0.02, *P* = 0.01 respectively). Median PFS was 593 days (CI 95%, 169–1016). Disease relapse after allo-HSCT was diagnosed in 19 (35%) pts. Causes of death were: relapse/progression (*n* = 13, 24%), infection (*n* = 10, 32%), hemorrhage (*n* = 4, 13%), “graft-versus-host” disease (*n* = 3, 10%), secondary malignancy (*n* = 1, 3%). Twenty three pts (43%) are still alive and in CR. HMA therapy may be an alternative approach for pts with AML, MDS, JMML, CMML as a “bridge” to allo-HSCT. Response to HMA therapy may impact EFS after allo-HSCT. Further larger prospective, randomized trials are needed for conclusions confirmation.

**Disclosure of conflict of interest:** None.

#### P682

##### **Elucidation of molecular markers for the diagnosis of chronic graft-vs-host disease developing after allogeneic hematopoietic stem cell transplantation**

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Chronic graft-vs-host disease (cGVHD) is the most troublesome complication developing after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Diagnosis of cGVHD has largely been based on clinical features only. We previously reported gene expression profiles in patients with cGVHD after allo-HSCT. We extended our study to develop a molecular diagnostic method of cGVHD. We selected six most commonly expressed genes from the former DNA expression study. And, a home-made 6-gene PCR array were used to evaluate gene expression profiles in the peripheral blood mononuclear cells of 39 patients given allo-HSCT (20 cGVHD patients, 19 non-cGVHD patients) and 19 normal controls. The gene expressions of the allo-transplanted patients were compared to those of the stem cell donors. SYBR green qPCR and multiplexqPCR were performed to confirm the usefulness of the selected genes in the diagnosis of cGVHD. InfoGainAttributeEal and Ranker were used to develop a gene model to diagnose cGVHD. k-Nearest Neighbor model and weka classifiers lazy IBk module were applied to evaluate the performance of the gene model. In another 21 steroid-refractory cGVHD patients (14 responders, 7 non-responders), the gene expression changes were analysed using our 6-gene PCR array before and 57 days after rituximab treatment. We identified six genes most accurately delineating cGVHD patients from those without

cGVHD; TGFB-induced factor homeobox 1, interleukin 2 receptor gamma, tetra trico peptide repeat domain 37, carbonic anhydrase I, serpin peptidase inhibitor clade A and MyoD family inhibitor. We established a 3-gene model (MyoD family inhibitor, TGFB-induced factor homeobox 1, tetra trico peptide repeat domain 37) to diagnose cGVHD. Our 3-gene model showed 81.00% sensitivity, 90.40% specificity, 80.8% precision, 81.03% accuracy and 86.90% ROC area in diagnosing cGVHD. TGFB-induced factor homeobox 1 increased in expression after rituximab treatment in responders. MyoD family inhibitor was found to be able to predict rituximab responses in steroid-refractory cGVHD patients. We could demonstrate that gene expression studies were useful in the diagnosis of cGVHD after allo-HSCT. We developed a 3-gene model to diagnose cGVHD.

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**Disclosure of conflict of interest:** None.

#### P683

##### Exergaming has an impact on quality of life after hematopoietic stem cell transplantation—a prospective, randomized study

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Hematopoietic stem cell transplantation (SCT) is physically and psychosocially demanding. However, exercise interventions may have positive impact on sentiment and psychological well-being in patients undergoing SCT. We report on a prospective, randomized study comparing the influence of a multimedia sensor-based practice with classical physiotherapeutic treatment (PT) on psychological aspects and quality of life (QOL). Patients undergoing SCT were randomized into the control group ( $n=23$ ) receiving PT or the experimental group exercising on the Nintendo-Wii ( $n=19$ ). Patients of both groups performed the exercises under the supervision of a physiotherapist and completed the Functional Assessment of Cancer Therapy - Bone Marrow Transplantation (FACT-BMT), Hospital Anxiety and Depression Scale (HADS-D) and Distress Thermometer at the date of hospital admission (T1) and on day 14 (T2), 28 (T3) and 100 (T4) after SCT. Questionnaires were completed by the participants independently and without supervision. Groups were compared using the Mann-Whitney U-Test. A  $P$  value  $<0.05$  was considered statistically significant. The median age of patients was 59 years in the control group and 57 years in the experimental group. Results of FACT-BMT generally showed a decline of the QOL domains measured on T2 and T3 and a raise at T4 in both groups. Physical well-being (PWB) showed the strongest fluctuation of all domains. It declined significantly between T1-T2 in both groups (PT  $P=0.015$ , Wii  $P=0.019$ ), followed by a significant increase between T2-T4 (both groups  $P=0.001$ ). However, only in Wii-group results of PWB at T4 ranked significantly above T1 ( $P=0.028$ ). Highest scores were proved for social and emotional well-being (SWB/EWB) in both groups. In Wii-group EWB increased significantly between T1-T4 ( $P=0.015$ ) and ranked above PT-group at all times. Functional well-being (FWB) scored lowest in both groups at all times. The score of bone marrow transplant scale (BMTS), the second lowest score in both groups, was always higher in Wii-group. The level of distress was comparable between both groups. However, at T2 distress increased above the cut-off level of 5 in both groups (Wii-group  $P=0.006$ , PT-group  $P=0.276$ ). This was accompanied by an increase of anxiety ( $P=0.705$ ) and depression ( $P=0.006$ ) in the PT-group, while both parameters decreased

in the Wii-group ( $P=0.087$  and  $P=0.220$ ), respectively. Anxiety in intervention group 5,8/4,4/5,0/4 at T1/T2/T3/T4 stayed below standard group 5,9/6,4/5,9/6,4 at all times. Depression averaged out at 4,9/6,5/5,4/5,7 in physiotherapy group and 5,5/4,3/5,9/3,8 in Wii-group. To the best of our knowledge, this is the first study to show that exergaming using the Nintendo-Wii is feasible in the immediate phase after HSCT. Exergaming may be regarded as beneficial since our data indicate less psychological distress and higher QOL in SCT recipients exercising with Nintendo-Wii. Therefore, it may be used in addition to PT.

**Disclosure of conflict of interest:** None.

#### P684

##### Expression of serum MicroRNAs is dysregulated during acute graft versus host disease

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Acute graft versus host disease (aGvHD) is the most frequent and serious complication following haematopoietic stem cell transplantation (HSCT), with a high mortality rate. A clearer understanding of the molecular pathogenesis may allow for improved therapeutic options or guide personalised prophylactic protocols. Circulating microRNAs are expressed in body fluids and have recently been associated with the etiology of aGvHD, but global expression profiling in a HSCT setting is lacking. This study profiled expression of  $n=799$  mature microRNAs in patient serum, using the NanoString platform, to identify microRNAs that were dysregulated at aGvHD diagnosis. Selected microRNAs ( $n=10$ ) were replicated in independent cohorts of serum samples taken at aGvHD diagnosis ( $n=42$ ) and prior to disease onset (day 14 post-HSCT,  $n=47$ ) to assess their prognostic potential. Sera from patients without aGvHD were used as controls. Dysregulated microRNAs were investigated in silico for predicted networks and mRNA targets. Profiling identified 61 microRNAs that were differentially expressed at aGvHD diagnosis. MiR-146a ( $P=0.03$ ), miR-30b-5p ( $P=0.007$ ), miR-374-5p ( $P=0.02$ ), miR-181a ( $P=0.03$ ), miR-20a ( $P=0.03$ ) and miR-15a ( $P=0.03$ ) were significantly verified in an independent cohort ( $n=42$ ). MiR-146a ( $P=0.01$ ), miR-20a ( $P=0.03$ ), miR-18 ( $P=0.03$ ), miR-19a ( $P=0.003$ ), miR-19b (0.02) and miR-451 ( $P=0.01$ ) were differentially expressed 14 days post-HSCT in patients who later developed aGvHD ( $n=47$ ). High miR-19b expression was associated with improved overall survival (OS) ( $P=0.008$ ), while high miR-20a and miR-30b-5p were associated with lower rates of non-relapse mortality ( $P=0.05$  and  $P=0.008$ ) and improved OS ( $P=0.016$  and  $P=0.021$ ). Pathway analysis associated the candidate microRNAs with haematological and inflammatory disease. Circulating biofluid microRNAs are dysregulated at aGvHD onset and have the capacity to act as prognostic and diagnostic biomarkers. Their differential expression in serum suggests a role for circulatory microRNAs in aGvHD pathology, which warrants further investigation.

**Disclosure of conflict of interest:** None.

#### P685

##### Factors associated with medication adherence amongst allogeneic hematopoietic stem cell transplantation recipients

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Treatments after allogeneic hematopoietic stem cell transplantation (HSCT) are long and constraining for patients. Medical adherence in HSCT patients is of major concern in daily practice but it has been not yet described.<sup>1,2</sup> The aims of our study were to evaluate treatment adherence and to identify factors associated with adherence behaviors. An observational single-center study was based on self-reported questionnaires completed by patients in a hematology day hospital between November 2015 and July 2016. The patient-reported adherence was evaluated using the eight-item Morisky Medication Adherence Scale (MMAS-8).<sup>3,4</sup> Individual item scores were summed: patients with a score of 8/8 were considered as good adherents to medication whereas a poor adherence referred to a score under 8. Among the latter, medium adherence ranged to a score of 6–8, while a score of <6 was considered low adherence. Socio-demographic and medical characteristics were collected by health records. A univariate model was used to evaluate if some of patients' characteristics were associated with adherence. Statistical analysis was performed using R software (Version 0.98.1103—2009–2014 RStudio, Inc). Fifty-six patients were included in the current study. Median age at transplantation was 55 years (range: 16–72 years). Diagnosis were AML (*n* = 28), ALL (*n* = 10), myelofibrosis (*n* = 8) and other hematological diseases (*n* = 10). 24 patients received a HSCT from a related donor (13 haploidentical). Myeloablative conditioning was used in 18 patients and reduced intensity regimen in 38 patients. A total of 64.3% (36/56) of the patients were poor adherent according to MMAS-8. Among these patients, 6/36 were low adherent and 30/36 were medium adherent. The results of univariate analysis showed that a poor adherence was associated with a longer time since HSCT and discharge at home. However elderly patients, patients treated with cyclosporine and patients with daily hydration at home were associated with a better adherence (*P* < 0.05). Our study presents the first data on adherence among patients undergoing HSCT. Risk factors associated with a poor adherence have been identified in order to determine patients' profiles that will benefit more from interventions to improve adherence. Particular attention has to be paid to younger patients. Efforts to establish a regular follow-up of these patients are needed in order to sustain patients in the treatment adherence to prevent the occurrence of severe complications.

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#### P686

### Feasibility and efficacy of high-dose chemotherapy and autologous hematopoietic cell transplantation for HIV-related lymphoma: A single-institution experience

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High dose therapy (HDT) followed by autologous hematopoietic cell transplantation (autoHCT) has been shown to be safe and effective in patients with HIV-related lymphoma (HRL). Data is limited to small case series, transplant registries and a single prospective multicenter observational study. Here we report our institutional experience with auto-HCT in patients with HRL. Twenty patients with HRL [non-Hodgkin = 14 (70%), Hodgkin = 6 (30%)] and treatable HIV infection underwent HDT consisting of carmustine, etoposide, cytarabine and melphalan (BEAM) followed by peripheral blood auto-HCT from 04/2006 to 07/2015. In 2 cases rituximab was administered as part of the preparative regimen. Patient-, disease-, and transplant-related characteristics are summarized in Table 1. Median age was 48 years (range: 35–61). The median follow-up for surviving patients was 42 months (range: 6–110). At transplant, median peripheral blood CD4 count was 226 cells/μl (range: 41–761). HIV viral load was undetectable in 14 out of 20 patients and lower than 4 logs in all of them. The median time to neutrophil and platelet engraftment were 11 days (range: 10–13) and 14 days (range: 13–176), respectively. Response rates at day +100 post-autografting in 17 evaluable patients were as follows: complete remission (CR) = 11/17 (65%), partial response (PR) = 2/17 (12%), and relapse/progression = 4/17 (24%). Median event-free survival (EFS) was 58.4 months. Median overall survival (OS) was 74.3 months. At 5-years post-transplantation, EFS and OS were 68% and 53%, respectively. Non-hematologic toxicities consisted of mucositis in 8 (grade 1 = 3, grade 2 = 5), and enteritis in 13 patients (grade 1 = 2, grade 2 = 3, and grade 3 = 8). There were 13 documented infections in 11 patients (bacterial = 9, viral = 2, fungal = 2). Six patients died from disease relapse/progression (*n* = 5) and infection (*n* = 1). Non-relapse mortality was 0% at day 100 and 5% at 5 years. Patients with HRL and treatable HIV infection should be offered autoHCT if indicated. HIV infection is no longer a contraindication for autoHCT in this population and outcomes appear to be similar to those seen in HIV negative patients.

#### [P686]

Table 1: Patient-, disease-, and treatment-related characteristics

Variables	N (%)
Total number of patients transplanted	20
Gender	Male=19 (90%) Female=2 (10%)
Age at time of auto-HCT median (range)	47(35-61)
Hodgkin's	6(30%)
Mixed cellularity	3
Nodular sclerosis	1
NOS	3
Non-Hodgkin's lymphoma	14(70%)
DLBC	7
Burkitt's	2
Plasmablastic	3
Primary effusion	2
Stage	
IIA	4(20%)
IIB	1(5%)
IIIA	1(5%)
IIIB	4(20%)
IVA	1(5%)
IVB	9(45%)
Median (range) number of previous lines of therapy*	2 (1-3)
Conditioning regimen	
BEAM	18 (90%)
BEAM+Rituximab	2 (10%)
Disease status at time of transplantation	
CR1	7(35%)
CR2	4(20%)
PR	8(40%)
PD	1(5%)

**Abbreviation:** N: Number of patients; M: male gender; auto-HCT: autologous hematopoietic cell transplant; NOS: not otherwise specified; DLBC: diffuse large B-cell lymphoma; BEAM: BCNU, etoposide, cytarabine (Ara-C), melphalan; CR1: first complete remission; CR2 :second complete remission; PR: partial response; PD:progressive disease. \*Unknown in 2 cases.

**Disclosure of conflict of interest:** None.

P687

**Fibroblast growth factor and dexamethasone have different effects on impaired immunosuppressive activity of mesenchymal stromal cells in lymphoma patients**

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The study of mesenchymal stromal cells (MSCs) in hemoblastoses reveals distinct changes in marrow stromal cell functioning [Perez-Simon JA, 2009; Bingzong L, 2010; Shipounova I, 2013; Shevela EY, 2010]. In particular, in patients with lymphomas MSCs was found to possess significantly reduced proliferative and immunosuppressive activities [Shevela EY, 2008]. A variety of mediators have a stimulating effect on the MSC proliferation - growth factors (PDGF, EGF, FGF-b, IGF-1) [Chen BY, 2012; Bianchi G., 2003], proinflammatory cytokines [Caplan A.I. 2007] and hormones [Xiao Y, 2010; Chen BY, 2012; Hong L, 2011]. However, the effects of these mediators on the immunosuppressive activity of MSCs remain virtually unexplored. We studied the effect of basic fibroblast growth factor (FGFb) and Dexamethasone on expansion and immune modulation of MSCs in patients with lymphomas. MSCs were generated from bone marrow aspirates obtained from the patients with Hodgkin's lymphoma (HL;  $n=8$ ) and non-Hodgkin's lymphoma (NHL;  $n=4$ ). The adherent fraction of marrow aspirate was cultivated with/without the basic fibroblast growth factor (FGF-b, 10 ng/ml) or Dexamethasone ( $10^{-5}$ M or  $10^{-8}$ M) to reach 80–90% confluence. Then MSCs were passaged with Accutase and used for experiments after 1–2 passages. The number of MSC precursors (CFU-F) in bone marrow of lymphoma patients was found to be significantly decreased both in patients with NHL ( $17 \pm 5$ ,  $P < 0.01$ ) or HL ( $26 \pm 5$ ,  $P < 0.05$ ). The time until 80–90% confluence was significantly increased and took on average  $26 \pm 2$  days (vs  $15.4 \pm 0.6$  in donors). Finally, the immunosuppressive ability of patient MSC was significantly lowered and was only registered at the high concentrations of MSCs (1:1 and 1:2). The expansion of patient MSCs was significantly promoted with FGFb resulting in a significant decrease of primary cell cultivation (from  $25.4 \pm 1.52$  to  $18.6 \pm 1.21$  days;  $P = 0.041$ ) and a statistically significant twofold increase in the number of cells received at the first passaging. In addition, in cultures with FGFb there was a decrease in the relative amount of resting MSCs and a threefold increase of cycled cells in CD73+ MSCs. Dexamethasone has also provided a moderate stimulating effect on the MSC growth. In fact, the use of  $10^{-5}$ M of Dexamethasone resulted in the increase of the cell yield by 1.6 times and of  $10^{-8}$ M—by 1.9 times. However, FGFb and Dexamethasone differed in their effect on the MSC ability to inhibit the proliferative response of T lymphocytes upon stimulation with mitogens or alloantigens. Indeed, FGFb failed to correct the impaired immunosuppressive activity of patient MSCs, and median percentage of suppression still remained lowered—17% vs 16% without FGFb. In contrast to FGFb, Dexamethasone could increase the immunosuppressive activity of patient MSCs by 1.5 times (in dose of  $10^{-5}$ M) and by 2.1 times (for  $10^{-8}$ M). Our data indicate that FGFb and Dexamethasone used during the generation of MSCs exert a stimulating effect on the MSC expansion. In contrast to FGFb, Dexamethasone, in the broad range: of doses, was able to enhance the suppressive properties of MSCs that are initially reduced in patients with lymphoma. These findings suggest the existence of at least two mechanisms of impairments in immunoregulatory function of MSCs in lymphomas—dependent and independent of the MSC proliferation.

**Disclosure of conflict of interest:** None.

P688

**Free nonabsorbable antibacterial digestive decontamination is associated with a low incidence of gastrointestinal acute GVHD and better GVHD-free/ relapse-free survival (GRFS) in the ATG-based conditioning regimens**

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Commonly antibacterial prophylaxis based of oral no absorbable antibiotic such as (neomycin colistin, gentamicin, vancomycin) used before and after engraftment, other fluoroquinolone such as levofloxacin were recently used to prevent invasive infection. However the exact interaction with gastrointestinal acute graft versus host disease (GI-aGVHD) remains unclear. The objective of this study was to evaluate a novel composite endpoint of GVHD-free/relapse-free survival (GRFS), in which events include grades 3–4 GI aGVHD, chronic GVHD requiring systemic therapy, relapse, or death in ATG based-conditioning regimens, with free no absorbable antibacterial digestive decontamination prophylaxis. A total of 39 evaluable consecutive patients with hematological disease were included in period of February 2013 to Mai 2016. Patients with malignancies disease ( $N=33$ ) received myeloablative conditioning regimens plus ATG (5 mg/kg); including once daily busulfan ( $130 \text{ mg/m}^2$ , -6d to -3d, iv) + fludarabine ( $40 \text{ mg/m}^2$ /d, -6d to -3d, iv) (AML = 26, ALL = 3, CML = 1) or Melphalan ( $140 \text{ mg/m}^2$ , d-1, iv) (ALL = 3). Six patients received Cy/ATG for SAA. GVH prophylaxis consisted to; ciclosporine A (CsA) + MTX. CsA was maintain levels between 150–400 ng/mL and tapered at the discretion of the treating physician. All patients were received peripheral blood stem cells (PBSC) graft from a matched related donor. Since December 2015 levofloxacin and voriconazole was administered as antibacterial and antifungal prophylaxis. Diagnostic, clinical grading and treatment of GI-aGVHD and GI-cGVHD were performed according to established criteria and NIH recommendations. Probability of GRFS was estimate by Kaplan–Meier method. Median age was 35 years (range: 6–60). Median dose of CD34+ and CD3+ cell doses were  $4.9 \times 10^6$  (range: 2.5–7) and  $1.84 \times 10^8$  (range: 0,036–5,28). The median time to neutrophil and platelet recovery were 13 days (range: 6–33) and 14 days (range: 10–43) respectively. At time of transplant 12/39 (31%) had an intestinal colonization with extended-spectrum beta-lactamase (ESBL) producing bacteria. Only 5/39 (13%) developed infectious diarrhea during the period of transplant. Incidence of grade III/IV GI-aGVHD and GI-cGVHD requiring systemic therapy were 5% and 5% respectively. For patients with malignancies diseases ( $N=33$ ), 23 (70%) were alive at a median follow up of 12 months (range: 5– 43). Incidence of relapse, disease free survival rates were 30%, 67% respectively. The GRFS rate as defined previously was 48% at 30 months. These results confirm that free no absorbable antibacterial digestive decontamination and ATG-based conditioning regimens were associated with very low incidence of GI-GVHD and better GRFS in patients with malignancies diseases. Diverse bacterial populations of the gastrointestinal tract remain important factors to promote immune tolerance after allogeneic SCT.

**Disclosure of conflict of interest:** None.

**P689**

**G-CSF primed HLA haploidentical transplantation from maternal or collateral donor using ATG plus reduced dose of posttransplantation cyclophosphamide: Results of a phase II prospective trial**

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The transplantation milieu using granulocyte colony-stimulating factor (G-CSF), and anti-thymocyte globulin (ATG) for HLA-haplotype-mismatched transplants from related donors has resulted in favourable outcomes with low transplant-related mortality (TRM), without increased relapse rate. However, in this transplant modality, the poorer outcome owing to high incidence of graft-versus host disease (GVHD) related to maternal donor or collateral donor remains a concern. Meanwhile the use of post-transplant cyclophosphamide (PT/Cy) in recent years appears to be protective against severe acute and chronic GVHD. We performed a prospective pilot study of HLA haploidentical stem cell transplantation (SCT) from maternal or collateral donors with intensified conditioning including G-CSF and ATG, followed by two lower doses of PT/Cy (14.5 mg/kg x 2 doses). Outcomes were compared with those of 160 controls from matched-pair analysis who undergone haploidentical SCT from other donors than mother or collateral relatives at the same time period. A total of 40 patients with myelodysplastic syndrome (MDS) or leukaemia undergoing haploidentical SCT from maternal or collateral donors were enrolled in the study. Incidence of grade II-IV and grade III-IV acute GVHD at day 100 were comparable between the study group and the control group (17.5% vs. 33.3%, *P*=0.07; 5.0% vs. 12.5%, *P*=0.24). Incidence of CMV and EBV reactivation at day 100 were also comparable between the study group and the control group (75.0% vs. 85.0%, *P*=0.16; 15.0% vs. 29.2%, *P*=0.09). After a median follow-up of 303 days and 341 days, the incidence of TRM and relapse at 1 year were comparable between the study group and the control group (5.0% vs. 13.3%, *P*=0.16; 10.0% vs. 6.7%, *P*=0.54); the probability of overall survival and LFS at 1 year were comparable between the study group and the control group (84.2% vs. 79.8%; *P*=0.24; 83.0% vs. 77.7%, *P*=0.48). In conclusion, conditioning with ATG and low-dose PT/Cy might be a feasible option for patients undergoing HLA haploidentical, T-cell replete SCT from maternal or collateral donors. Trial registration: The study is registered at www.clinicaltrials.gov as NCT02412423.

**Disclosure of conflict of interest:** None.

**P690**

**Hematopoietic stem cell transplantation comorbidity index in transplant recipients and impact of pulmonary and cardiac function values on its score**

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Hematopoietic stem cell transplantation (HCT) is a lifesaving treatment option for eligible patients with hematological malignancies. HCT is inherently associated with a risk of non-relapse mortality that varies greatly depending on transplant and patient characteristics. The assessment of the risk of complications and mortality before the procedure is extremely important. The HCT Comorbidity Index (HCT-CI) introduced by Sorror M. is one of the tools proved to predict HCT outcomes and was shown to be significant in various disease and HCT settings. The objective is to evaluate HCT-CI index of HCT recipients, determine impact of different variables on CI score, particularly those, showing pulmonary and cardiac function. Data of HCT-CI of autologous (Auto) and allogeneic (Allo) HCT recipients, transplanted during period January 2015–October 2016 were analyzed. Impact of pulmonary and cardiac function

values on CI score was evaluated: DLCO (diffusing capacity of the lung for carbon monoxide), FEV1 (forced expiratory volume) and EF (cardiac ejection fraction) are parameters, reflecting pulmonary and cardiac function, which values are included into HCT-CI score. The statistical data analysis was conducted using SPSS program. The differences were considered statistically significant at *p* ≤ 0.05. Records of 100 allo and 220 auto HCT recipients, transplanted during 01.2015 – 10.2016 in Vilnius University Hospital were revised. Median age of allo HCT and auto HCT recipients was 50 (19–75) and 58 (19–74) years respectively. Main indication for allo HCT was acute myeloid leukemia 48 (48%) patients and for auto HCT - multiple myeloma 133 (60.5%) patients. HCT-CI was completely calculated (no values missing) in 64 allo and 102 auto HCT recipients. Only patients with available complete HCT-CI data were further analyzed. HCT-CI in HCT recipients was as shown in Table 1. HCT-CI score < 3 was calculated in 37 (57.8%) and ≥ 3 in 27 (42.2%) allo HCT recipients. HCT-CI score < 3 was calculated in 45 (44.1%) and ≥ 3 in 57 (55.9%) auto HCT recipients. HCT-CI score did not differ statistically significant between male and female recipients in both HCT categories as well as in different age groups of patients (below and above 40 years in allo and below and above 60 years in auto HCT). DLCO was found to be below normal values (< 80%) in 43 (67.19%) allo HCT and in 67 (65.7%) auto HCT recipients. FEV1 was less affected and found to be lower 80% in 6 (9.3%) allo HCT and in 17 (16.7%) auto HCT recipients. EF below 50% detected in 1 (1.6%) allo HCT and in 6 (5.9%) auto HCT recipients. Low DLCO was found to cause the greatest impact on HCT-CI score and was statistically significantly associated with higher HCT-CI (*P* < 0.001). The most common HCT-CI in both HCT groups was score 3. DLCO was found to be below normal ranges in relatively large patient group and had the greatest impact on HCT-CI score. Further studies on reasons of pulmonary function impairment and its impact on HCT outcomes are warranted.

[P690]

**Table 1. HCT-CI of HCT recipients**

HCT-CI	Allogeneic HCT	Autologous HCT
0	13 (20,3 %)	23 (22,5 %)
1	8 (12,5 %)	7 (6,9 %)
2	16 (25 %)	15 (14,7 %)
3	19 (29,7 %)	37 (36,3 %)
4	3 (4,7 %)	14 (13,7 %)
5	2 (3,1 %)	5 (4,9 %)
6	3 (4,7 %)	1 (1 %)

**Disclosure of conflict of interest:** None.

**P691**

**Hematopoietic stem cell transplantation for haemophagocytic lymphohistiocytosis: A single-center report of 61 patients**

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Haemophagocytic lymphohistiocytosis (HLH), a life-threatening hyper-inflammation syndrome, is classified into primary and secondary forms. Primary HLH is caused by gene mutations resulting in impaired cytotoxicity of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). Secondary HLH arises in the setting of autoimmunity, infection, malignancy, or less commonly, may be idiopathic. Treatment of HLH has two major goals: Halting the triggering event and controlling the overactive immune system. However, patients with primary or recurrent secondary HLH should subsequently undergo allogeneic HCT for long lasting disease remission. We retrospectively evaluated 61

patients with median ages of 23 years (range: 11–54 years) between December 2006 and March 2016, which including 7 case of primary HLH (Homozygous missense mutation in UNC13D:  $n=3$ ; Homozygous missense mutation in PRF1:  $n=1$ ; Heterozygous missense mutation in PRF1 in the combination with hemizygous missense mutation in SH2D1A:  $n=1$ ; Mutation in RAB27A:  $n=1$ ; Mutation in ITK:  $n=1$ ). 5 cases of unknown causes HLH, 10 cases of lymphoma-HLH (NK/T-cell lymphoma:  $n=6$ , Primary  $\gamma\delta$ T cell lymphoma in skin:  $n=1$ ; Subcutaneous panniculitis-like T cell lymphoma:  $n=2$ ; Primary T cell lymphoma in skin:  $n=1$ ) and 39 cases of EBV associated HLH. 41 patients achieved CR+PR before HSCT, and 20 patients NR. 47 patients were transplanted from HLA-haploidentical family donors, 13 from HLA-identical sibling donors, and 1 from a matched unrelated donor. Conditioning regimen include TBI and Non-TBI. The median overall survival rate was 65.6% with a median survival time of 38 months (range: 5–119 months). OS of Primary HLH is 85.7%, OS of unknown causes HLH is 60%, OS of lymphoma-associated HLH is 60%, OS of EBV-HLH is 64.1%. OS of CR+PR is 80.5%, OS of NR is 35.0% 6 patients without engraftment died because of 2 graft failure and 4 toxicity of conditioning regimen. 15 patients with engraftment died. Of those, 1 patient died of HSCT-associated TMA, 3 patient died of grade IV aGVDH, 5 patients died of relapsed HLH or organ failure as results from unsuccessful treatment of the progressively elevated EBV-DNA load. 2 patient died of tumor relapse, and 4 patient died of infection. Acute GVDH occurred in 42 patients with grade I-II aGVDH in 25 patients and grade III-IV aGVDH in 17 patients; chronic GVDH occurred in 19 patients. 46 patients achieved completed chimerism, 9 patients appeared with mixed chimerism, and 2 patient presented with graft failure. Of 34 EBV-HLH with engraftment, reactivated EBV infection was found in 33 (97%) with the whole blood EBV-DNA load at 103–107 copy numbers per ml. PTLD occurred in 3 patients confirmed by pathology. After reduced immunosuppressors, negative result of EBV infection was obtained while patients developed GVDH. For 39 EBV-HLH, Patients who carry with EBV loading EBVDNA  $\leq 105$  copies/ml before transplantation, overall survival rate was significantly higher than that of EBVDNA  $> 105$  copies/ml ( $P < 0.05$ ); who achieved CR+PR OS was significantly higher than that of NR ( $P < 0.05$ ); who range: from diagnosis to transplantation  $\leq 6$  months OS was significantly higher than that of  $> 6$  months ( $P < 0.05$ ). Allogeneic hematopoietic stem cell is an effective method for primary HLH and lymphoma-HLH, EBV-HLH, even haploid transplantation. The remission status before transplantation is decisive for the prognosis.

**Disclosure of conflict of interest:** None.

#### P692

### Hepatic veno-occlusive disease after allogeneic hematopoietic stem cell transplantation in a single centre: Revised diagnosis and incidence according to new ebmt classification

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Sinusoidal obstruction syndrome, also known as veno-occlusive disease (SOS/VOD), is a potentially life threatening complication that can develop early after hematopoietic cell transplantation (HCT). In this study we retrospectively investigated the incidence, risk factors and outcomes of SOS/VOD in 978 transplants, performed in 896 patients between march 1982 and may 2016, on the basis of the new diagnostic criteria and classification of the EBMT. The patient's median age was 31 years (1 to 71). Of them, 536 were males and 442 females. 896 patients received one transplant and 82 two transplants. A diagnosis of hematological malignant and nonmalignant disease was present in 784 and 194 cases, respectively. The disease risk at HCT was standard in 397 cases, intermediate in 237 and high in 344. An HLA identical sibling donor was used in 691 cases, an unrelated donor in 166 and a haploidentical

family donor in 121. Conditioning was myeloablative in 813 transplants and at reduced intensity in 165. Source of hematopoietic stem cells was bone marrow in 680 transplants and peripheral blood in 298. We did not limit the diagnosis of SOS/VOD to the classical 21 days after HCT, but all suspicious cases appearing in the first 100 days were evaluated. SOS/VOD was diagnosed in 56 cases, of which 47 in the first 21 days after transplant and 9 between day 22 and 50 (median day 9). Their main clinical characteristics are shown in Table 1. The severity of SOS/VOD was mild in 5 patients (9%), moderate in 6 (11%), severe in 8 (14%) and very severe in 37 (66%). The cumulative incidence (CI) of SOS/VOD was  $5.7 \pm 0.005\%$ . Among the most relevant variables studied in univariate analysis (recipient age and gender, ferritin level at HCT, type of hematological disease, disease risk at HCT, type of donor, number of transplants, time of transplant, drugs used in the conditioning regimen, intensity of the conditioning regimen, source of stem cells), there was no factor with an adverse impact on SOS/VOD incidence. Of 56 patients with diagnosis of SOS/VOD, 41 (73%) died. SOS/VOD was the main cause of death in 9 patients and a relevant contributing cause of death in 10. Of relevance, 6 of 8 patients (75%) with severe SOS/VOD and 34 of 37 patients (92%) with very severe SOS/VOD died, whereas only one patient with moderate SOS/VOD died and no patient with mild SOS/VOD died. Among 56 patients with SOS/VOD, 19 received defibrotide therapy and 37 the best supportive available therapy. Defibrotide was given for a median of 20 consecutive days (range: 5 to 87), starting at day 18 post-HCT (range: 3 to 49) with a median total bilirubin level of 3,16 mg/dl (range: 1.4–20.7). The 1-year overall survival (OS) of patients treated with defibrotide was better as compared to that of patients who received the supportive therapy (47% versus 27%) although the difference doesn't reach the significance ( $P=0.25$ ). The occurrence of SOS/VOD does influence significantly the 1-yr OS considering that it was 72 +1.5% for patients without SOS/VOD and 33+6% for patients with SOS/VOD ( $P=0.0001$ ). In conclusion, the new EBMT diagnostic and severity criteria for SOS/VOD has been very useful in identifying patients with severe and very severe forms of this complication. If validated in prospective studies, these criteria will allow an earlier selection of patients requiring immediate therapeutic intervention.

[P692]

Table 1. Main characteristics of 56 SOS/VOD cases

Hyperbilirubinemia $> 2$ mg/dL	56	100%
Bilirubin doubling in 48 hours	34	60%
Maximum level of bilirubin, median (range)	7,7 mg/dL	(2-50)
Ascites	24	43%
Weight gain $> 5\%$	32	57%
Painful hepatomegaly	40	71%
AST increase	34	61%
ALT increase	40	71%
Renal function impairment	28	50%
Dialysis / Hemofiltration	5	9%

**Disclosure of conflict of interest:** None.

#### P693

### High dose chemotherapy and autologous stem cell transplantation for patients with high risk or relapsed Ewing sarcoma family of tumors: a single institute experience

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The prognosis of patients with newly diagnosed Ewing's sarcoma family of tumors (ESFT) has improved significantly over the last few decades. Nonetheless, the long-term survival is still below 35% patients with high risk features. The role of

high dose chemotherapy and autologous stem cell transplantation (HDCT and ASCT) for high risk and relapsed ESFT was analyzed. A retrospective medical chart review was done on patients with EFST who underwent HDCT and ASCT between September 1998 and January 2015 at Seoul National University Children's Hospital. Indications for HDCT and ASCT included metastasis at diagnosis, bulky primary tumor (> 100 mL), axial/central primary site, and relapsed disease. Single HDCT and ASCT was performed in the earlier period, and the regimen was changed from MEC (melphalan, etoposide, carboplatin), to Topothiocarbo (topotecan, thiotepea, carboplatin), and to BuMel (busulfan, melphalan). Tandem HDCT and ASCT was performed in the recent period, 1st HDCT with BuMel and 2nd HDCT with modified MEC (melphalan, etoposide, carboplatin). Twenty-one patients who were diagnosed with ESFT at a median age of 8.7 years old underwent conventional chemotherapy, radiation therapy and/or surgery and received HDCT and ASCT in complete response ( $n=14$ ) or partial response ( $n=7$ ). The overall survival of the patients was 70.0% at median 3.6 years and the event free survival (EFS) of the patients was 55.0% at median 2.9 years from the last ASCT. The EFS of the patients who underwent single HDCT and ASCT with MEC ( $n=11$ ), Topothiocarbo ( $n=1$ ), and BuMel ( $n=4$ ) was 54.5%, 0.0% and 75% respectively. The EFS of the patients who

underwent tandem HDCT and ASCT ( $n=5$ ) was 50.0%. Seven patients relapsed at median 6.6 months from the last ASCT. Despite further treatment, 5 patients died of disease and 2 patients are currently alive without disease. One patient developed acute myeloid leukemia at 17.8 months from the last ASCT and is currently alive without disease after additional chemotherapy, HLA-haploidentical stem cell transplantation and donor lymphocyte infusions. One patient died of transplantation-related mortality due to septic shock and lung infection. HDCT and ASCT may be a promising treatment option for patients with high risk or relapsed ESFT. Further refinements may be needed to identify the optimal regimen and number of HDCT and ASCT.

**Disclosure of conflict of interest:** None.

**P694**

**High incidence of engraftment syndrome in haploidentical transplant patients with the use of post transplant Cyclophosphamide in Chile**

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[P694]

Table 1: Patients, donors, conditioning and graft characteristics

Haploidentical (N = 22)	
Age, Median (years)	33 (14-62)
<i>Diagnostics (N,%)</i>	
AML	10 (45.4%)
ALL	4 (18.1%)
Lymphomas	4 (18.1%)
Myeloproliferative	3 (13.6%)
Aplastic Anemia	1 (4.5%)
<i>Conditioning (N,%)</i>	
Myeloablative	14 (63.6%)
Cy120 / TBI 1200	13 (59.0%)
Cy60 / Flu120 / Busulphan 9.6mg / kg	1 (4.5%)
Non-myeloablative	8 (36.0%)
Cy60 / Flu120 / TBI 200	7 (31.8%)
Flu120 / Mel140	1 (4.5%)
<i>Stem Cell Origin</i>	
Bone marrow	1 (4.5%)
Peripheral Blood	21 (95.4%)
CD34 + ( $10^6$ / kg) median,range	5.75 (2.5-13.4)
<i>HLA Match (-A, -B and -DR) intermediate resolution</i>	
3/6	18(81.8%)
4/6	3 (13.6%)
5/6	1 (4.5%)
<i>Gender Donor-host, respectively (N,%)</i>	
Male-Male	10 (45.4%)
Female-Male	3 (13.6%)
Male-Female	7 (31.8%)
Female-Female	2 (9.0%)

Table 2: Engraftment syndrome (ES) symptoms

Symptoms	N (%)
N	9
Fever $\geq 38^\circ\text{C}$	8/9 (89%)
Rash > 25 % BSA	8/9 (89%)
Hypoxemia	2/9(22%)
Total bilirubin > 2 mg / dL	1/9 (11%)
Creatinine > 2 mg / dL	3/9 (33%)
Weight gain > 2.5 % from baseline	2/9 (22%)
Encephalopathy	-
Systemic corticosteroids	6/9 (67%)
No Systemic Corticosteroids	3/9 (33%)

Post Transplant Cyclophosphamide (PT-Cy) has expanded the use of unmanipulated haploidentical grafts which have a high HLA disparity between host and donor. One of the consequences of HLA disparity is the development of engraftment syndrome (ES). This is an immunological reaction characterized by non-infectious fever and skin erythema that develops after neutrophil engraftment. ES resembles an infectious process but treatment involves the use of high dose steroids. Our hypothesis is that pts undergoing haploidentical transplants (HAPLO) with PT-Cy should have a high rate of ES given the high HLA disparity between donor and recipient. Objectives: To determine the incidence, symptoms, morbidity and mortality of ES in patients undergoing HAPLO with PT-Cy at our institution. Retrospective analysis of 22 patients with high-risk hematological diseases undergoing HAPLO with PT-Cy at Clinica Santa Maria between November 2012 and August 2016. ES was diagnosed using the Spitzer criteria (1). ES was diagnosed if pts met 3 major criteria OR 2 major plus 1 minor criterion. Symptoms could occur prior to or after neutrophil engraftment (neutrophils over 500 cells / uL). All patients signed informed consent and the study was reviewed by our institutional review board. 22 patients received haploidentical grafts (Table 1). All patients had neutrophil engraftment at a median of 18 days. 9/22 patients (41%) had symptoms that met criteria for ES (Table 2). 2/9 were transferred to ICU due to hypoxemia and 1 patient died after diagnosis of ES. 5/9 pts were treated steroids. All patients received broad spectrum antibiotics during the febrile period and neutropenia. Blood cultures, EBV and CMV PCR were negative in all ES pts. There were no significant differences in hospital stay or one-year overall survival (OS) between patients who developed and pts who did not develop ES (median 37 vs. 35 days respectively,  $P = 0.68$ ; one-year OS 56% vs. 57%,  $P = 0.86$ , respectively). ES is a frequent complication in patients undergoing HSCT haplo with PT-Cy. The incidence of ES in our study was higher when compared to historical full match related donors series and lower when compared to cord blood transplant studies (2) There was no increased morbidity and mortality associated with ES diagnosis. Prompt institution of steroids is recommended in ES patients after ruling out an underlying infectious process to avoid further complications.

#### References

1. Spitzer *et al.* *Bone Marrow Transplant* 2001; **27**: 893–898.
2. Spitzer *et al.* *Bone Marrow Transplant* 2015; **50**: 469–475.

**Disclosure of conflict of interest:** None.

#### P695

##### HLA-haploidentical transplantation using T-cell-replete grafts and high-dose cyclophosphamide post-transplantation in the treatment of relapsed/refractory T-cell lymphoma/leukemia

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Hematopoietic stem cell transplantation (HSCT) might be a valid treatment option for adults suffering from aggressive T-cell malignancies providing long term disease control. Since a suitable HLA-matched donor cannot be identified for all patients (pts) in need for transplantation, alternative donors graft sources such as related HLA-haploidentical donors are considered. Through introduction of T-cell-replete (TCR) HLA-haploidentical transplantation (haplo-HSCT) using post transplantation cyclophosphamide (PTCY) successful treatment with low non-relapse mortality rate (NRM) has been observed in lymphoma patients (Luznik *et al.*, BMT, 2008). However, less

data are available on the outcome of this haplo-approach in the treatment of T-cell malignancies, in particular when disease is refractory. We retrospectively evaluated the outcome of haplo-HSCT using TCR grafts and PTCY in 8 pts with peripheral T-cell lymphoma treated between 2010 and 2015 at our institution (T-NHL = 6, T-ALL = 2; male  $n = 5$ ; median age: 37 years). Disease was refractory/active at time of transplantation in 7 pts, while one had achieved second CR. All patients received at least 2 prior treatment lines and one patient failed previous allogeneic transplantation. While fludarabine and cyclophosphamide served as backbone for conditioning, 3 pts received a TBI-based and 5 a drug-based conditioning regimen which was myeloablative in 50%. If disease was active at time of haplo-HSCT, a sequential therapeutic concept was performed involving intensive chemotherapy (clofarabine  $n = 6$ ) shortly preceding conditioning (Zoellner AK *et al.*, BMT, 2015). Post-grafting immunosuppression consisted of cyclophosphamide, tacrolimus and mycophenolate mofetil in all patients. Graft source was bone marrow in 3 pts. No primary graft rejection occurred; 7/8 pts engrafted, one died early in aplasia. Neutrophil/platelet engraftment was achieved at a median of 20 (range: 14–36) and 42 (range: 17–117) days, respectively. Acute GvHD grade II–III was observed in 4 pts, whereas no patient developed grade IV aGvHD. Mild chronic GvHD occurred in one patient. 50% of the pts developed grade II–III treatment-related toxicities most commonly diarrhea (33%) and mucositis (25%); grade IV toxicity (mucositis) was observed in one patient only. No VOD occurred. CMV reactivated in 4/5 pts at risk, whereas no PTLD was seen. Proven invasive aspergillosis was diagnosed in one patient. At day +30 seven pts achieved CR. 3 pts relapsed and 3 died (relapse  $n = 1$ , infection  $n = 2$ ). 1-year NRM was 25%. At a median follow up of 46 months (range: 15–76) the estimated 1-year and 3-year overall survival (OS) and progression-free survival (PFS) were 63%/63% and 50%/33%, respectively. Three pts received haploidentical DLT pre-emptively ( $n = 2$ ) and therapeutic ( $n = 1$ ), leading to sustained CR in two, while no severe GvHD occurred. Sequential therapy in the setting of TCR haplo-HSCT using PTCY as GvHD prophylaxis is feasible, well tolerated and shows low rates of GvHD and acceptable NRM in patients with relapsed/refractory T-cell lymphoma/leukemia providing an effective anti-lymphoma/leukemic activity. Thus, we suggest that intensified TCR haplo-HSCT using PTCY should be considered as an alternative for patients suffering from aggressive T-cell malignancies, lacking HLA-matched donors.

**Disclosure of conflict of interest:** None.

#### P696

##### Incidence of secondary primary malignancies (SPM) in patients with multiple myeloma (CALM study)

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As the outcome of multiple myeloma (MM) patients (pts) continues to improve, development of late complications particularly SPM are of concerns. We examined the incidence of SPM in MM pts who were enrolled in the prospective EBMT CALM study (Collaboration to Collect Autologous Transplant outcome in Lymphoma and Myeloma). A total of 3757 pts with MM were enrolled and underwent first autologous hematopoietic stem cell transplant (HSCT). Pts characteristics are as follows: median age 59 year (19–77), gender M/F 2180/1577, subtype IgG 2028 (54%), IgA 714 (19%), IgM 21 (0.6%), lines of induction regimens prior to HSCT one in 2003 pts (53%), two in 724 pts (19.3%), > 2 in 348 pts (9.3%), and missing in 682 pts (18%). Induction regimens included IMiDs and proteasome inhibitor (PI)s with alkylating agents in 1266 pts (33.7%), IMiDs and PIs with no alkylating agents in 1328 (35.5%), and alkylating agents with no IMiDs or PIs in 478 (12.7%) and missing data in 685 (18%). Radiotherapy was used pre HSCT in 614 pts (16.3%), no radiation in 2461 pts (66%) and missing data in 682 (18.2%). Plerixafor (P) was administered mostly for poor HSC mobilization as defined by the centers in 285 pts (7.6%), 3373 pts (90%) did not get plerixafor, and data are missing in 99 pts (2.6%). Disease status at HSCT; CR/VGPR in 1664 pts (44.3%), PR in 1721 (46%), < 12 mo in 2958 pts (79%) and > 12 mo 799 pts (21%). Conditioning regimen was mainly melphalan in 3659 pts (97.4%), and melphalan with other drugs in 75 pts (2.0%). KARNOFSKY PERFORMANCE STATUS > 90% was documented in 2326 pts (62%) and < 90% in 1086 pts (29%). Number of HSC collected <  $3 \times 10^6$  in 239 pts (6.4%), 3–5 in 397 pts (10.6%), >  $5 \times 10^6$  in 1394 pts (37%), and data missing in 1727 (46%). The number of CD 34+ HSC infused <  $3 \times 10^6$  in 760 pts (20%), 3– $5 \times 10^6$  in 1055 pts (28%), >  $5 \times 10^6$  in 799 pts (21%), and missing in 1143 (30%). A total of 141 pts developed SPM with cumulative incidence of 5.4% (95%CI 4.4,6.3) at 72 mo. Data are missing in 414 pts (11%). Median time to development of SPM is 33 mo. (2.1–86.5) with 75% occurring in the first 50 mo. Ninety nine pts developed solid tumors and 31 hematologic malignancies and unknown type in 11. Overall survival for the whole group is 65.4% (63–67) at 5 yr post auto transplant, and 38% (25–52%) at 5 yr post-SPM in pts who developed SPM. Use of radiotherapy, type of induction, HSC cell dose did not influence the cumulative incidence of SPM. The incidence of SPM in this large prospective study is 5.4% at 72 months and is comparable to the reported incidence of SPM in the literature.

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## P697

### Interaction between center effect and strategy for GVHD prophylaxis on outcome of T-cell depleted and T-cell replete haploidentical transplant: An analysis on behalf of ALWP-EBMT

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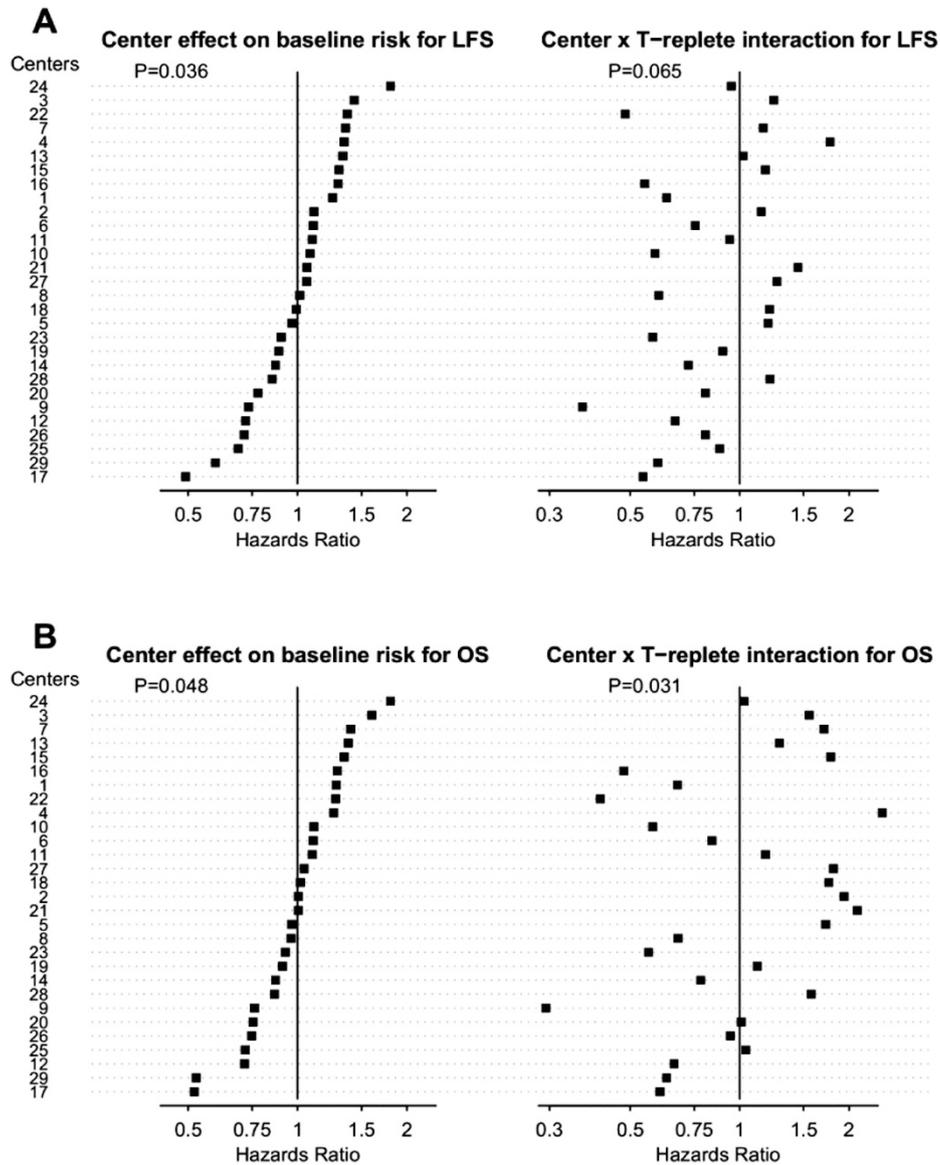
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Haploidentical allogeneic hematopoietic stem cell transplants (haplo-HSCT) is an alternative transplant procedure for patients with hematologic malignancies that are in need of transplant and do not have a compatible donor. Due to the broad HLA disparity, the haplo-HSCT can be performed with T-cell depletion and megadose of CD34+. Alternatively haplo-HSCT can be performed with non T-cell depleted transplants (T-replete) either in combination with anti-thymoglobuline serum (ATG) or post-transplant cyclophosphamide (PT-Cy) as GVHD prophylaxis strategy. Center effect is a known risk factor for outcomes of haplo-HSCT in both T-cell depleted (TCD) and T-replete settings. However, many centers tend to specialize in one GVHD prophylaxis strategy making it difficult to differentiate the treatment effect from the center effect. The objective was to investigate the role of center effects in GVHD prevention strategy, on leukemia-free survival (LFS) and overall survival (OS) in a population of adult patients with acute leukemia receiving haplo-HSCT. A retrospective multicenter study was conducted on patients reported to the EBMT registry. Inclusion criteria were: age > 18 years, lymphoblastic or myeloid acute leukemia (ALL or AML) in first or second complete remission (CR1 or CR2), receiving a haplo-HSCT between 2005 and 2014. In this population ( $n=606$ ), in order to assess the interaction between center and GVHD prevention treatment, we then included in the study selected patients from the centers that had performed more than 20% of both TCD and T-replete haplo-HSCT during the study period. Center effects on the outcomes consisted of 1) center effect on the baseline risk of event and 2) interaction between center and strategy of GVHD prevention. These center effects were estimated using Cox mixed-effects models and tested using permutation tests. All models were adjusted on age, CMV statuses, disease (ALL or AML), secondary leukemia, previous autologous transplant, disease status (CR1 or CR2), peripheral blood vs. bone marrow transplant, conditioning regimen. After selection, 226 patients were available across 29 centers in Europe. One hundred and one (45%) patients received TCD, 125 T-replete haplo-HSCT (62 (27%) using ATG and 63 (28%) using PT-Cy). Overall, 175 (77%) patients had AML. There were 86 (69%) peripheral blood transplants in the TCD group and 92 (91%) in T-replete. Median follow-up was 2.7 years. In adjusted analyses, without accounting for center effect, T-replete tended to be associated with better LFS (Hazard Ratio (HR): 0.70 (95%CI 0.45–1.07),  $P=0.10$ ) and OS (HR=0.67 (95%CI 0.43–1.04),  $P=0.076$ ). When center effects were included, there was significant heterogeneity across centers on the baseline risk of both outcomes (LFS:  $P=0.036$  and OS:  $P=0.048$ ). When accounting for interaction between center by strategy for GVHD prevention, the effect of T-replete vs. TCD on the outcomes did vary across centers ( $P=0.065$  and  $P=0.031$  for interactions in LFS and OS, respectively) (Figure 1). We found an interaction between center and strategy for GVHD prevention on outcomes of patients who received a haplo-HSCT. The difference between the 2 strategies (TCD or T-replete) varied across centers, in size and direction. This could be in part related to the increase in expertise with each technique in some centers and with the different management of complications, such as infections-related and relapse.

**Disclosure of conflict of interest:** None.

**Figure 1:** Center effects on leukemia-free survival (LFS, panel A) and overall survival (OS, panel B)



**P698**

**Introducing TacroCalc: A tacrolimus dose calculator for android and iOS that promotes therapeutic tacrolimus dosing post allogeneic stem cell transplantation**

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Tacrolimus is a calcineurin inhibitor increasingly used as immunosuppression following allogeneic stem cell transplantation; maintenance of therapeutic serum levels is essential to reduce the risk of graft rejection and graft versus host disease. However, tacrolimus can be associated with serious side effects and potential drug interactions. Regular monitoring of serum levels and appropriate dose adjustment is essential to ensure therapeutic levels and to avoid toxicity. In our adult

BMT unit, an established standard operating procedure (SOP) provides a prescriptive dosing algorithm for: (i) initiation of tacrolimus therapy; (ii) conversion between IV and oral routes; (iii) dose adjustment based upon tacrolimus serum level and interacting medications. We performed an audit assessing adherence to the SOP dosing algorithm. 97 inpatient tacrolimus dosing episodes from five consecutive haploidentical transplants were retrospectively analysed. 17 episodes were excluded due to insufficient records. For the remaining 80 episodes, tacrolimus serum levels and corresponding doses were identified. The response of the medical team to each serum level was compared with the SOP dosing recommendation. To account for sensible rounding of doses, a margin of error of  $\pm 10\%$  was permitted. Adherence to SOP dosing was 54%. Non-adherence to the SOP (46%) was subcategorized as justifiable (21%) or unjustifiable (25%). Justifiable non-

adherence included recognition of spuriously high levels (typically from contaminated lines) and delayed dose adjustment due to late reporting of levels by the laboratory. The most common cause of unjustifiable non-adherence was failure to increase the dose in response to a low level. Inadequate or excessive dose adjustments may be due to lack of experience or unfamiliarity with the SOP. Two interventions were launched with the aim of improving adherence to the SOP for therapeutic tacrolimus dosing. Firstly, to provide a rapid and user-friendly calculation method, we developed a mobile phone application (tacroCalc, a dose calculator based upon the SOP algorithm) for android and iOS devices using Python and Swift, respectively. Secondly, to reduce the number of spuriously high levels, all nurses responsible for specimen collection participated in an educational module delivered by medical and senior nursing staff. Key messages included the need to: use only the dedicated colour-coded tacrolimus lumen to infuse IV tacrolimus; avoid sampling from this lumen; sample peripherally when other lumens are known to be contaminated (reasons for this are being explored); suspend infusion of IV tacrolimus 15 minutes before taking a level; send only immediately pre-dose levels for oral tacrolimus. Initial re-audit of 16 episodes post intervention (data collection is ongoing) demonstrated a 40% increase in SOP adherence ( $P=0.03$ ; Fisher's exact test), with no cases of unjustifiable non-adherence and a significant reduction in spuriously high levels. In conclusion, the use of tacroCalc by doctors and the implementation of targeted teaching for nurses dramatically improved adherence to the tacrolimus SOP. This should ultimately improve therapeutic dosing whilst avoiding toxicity, which may result in better transplant outcomes. tacroCalc is now being adapted to include an option for paediatric dosing, with the potential to dose related medications such as cyclosporine.

**Disclosure of conflict of interest:** None.

#### P699

##### **Investigating frequency of EBV reactivation following autologous HSCT in patients with multiple sclerosis**

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Autologous HSCT is currently being explored for its efficacy and safety in the treatment of Multiple Sclerosis (MS). As more experience is gained in treating this cohort, treatment related mortality has steadily improved although the procedure still carries a degree of risk. EBV reactivation is well described in allogeneic stem cell transplants although less so in autologous transplantation. We investigated the frequency of EBV reactivation in patients with MS undergoing autologous HSCT at a single UK site. 30 patients underwent autologous HSCT for treatment of MS at King's College Hospital between Feb 2012 and Aug 2016. All were mobilised with Cyclophosphamide 4g/m<sup>2</sup> and G-CSF. 29 were conditioned with cyclophosphamide and ATG, and one with BEAM/ATG. Previous exposure to EBV (EBV IgG) was assessed prior to transplant and local post-transplant EBV monitoring was performed on whole blood samples by means of quantitative PCR in 26 patients. Data was collected retrospectively. All 26 (100%) patients were positive for EBV IgG pre-transplant. Overall, 194 samples were tested for monitoring post-transplant. 20 (76.9%) patients demonstrated positive PCR post-transplant on local testing with one further patient being negative on local tests but later becoming positive on testing in their parent hospital (full results unavailable). Of these 20, the median time to positive testing post-transplant was 24 days (7–91). Maximal EBV DNA titre was reached at a median time of 40 days post-transplant (7–101) with a mean maximum titre of 5.17 log (3.3–8.2). 2 patients experienced symptomatic reactivation with an associated large paraproteinemia. One of these developed hyper-viscosity requiring plasma exchange and developed

neurological symptoms mimicking an MS relapse (max EBV titre of 8.2 log). This patient received Rituximab and EBV level is declining, the other was observed carefully but developed right leg weakness which is slowly improving. The patient with raised EBV at their parent hospital also received rituximab (unclear if this reactivation was symptomatic). We have developed a protocol to pre-emptively treat EBV reactivation with Rituximab once a 6 log titre is reached and one patient has so far been treated according to this. Of the 18 patients with locally confirmed reactivation who did not receive Rituximab, 9 (50%) self-resolved at a median time of 98 days (44–182), 5 (33.3%) have ongoing re-activation (4 with improving, 1 with stable titres) and 4 (22.2%) have not had any local bloods performed  $\geq$  6 months. 1 patient with self-limiting reactivation later had a further positive titre (370 days post-transplant and 182 days post initial resolution). EBV reactivation appears to be common in patients with MS in the first 3 months post autologous HSCT. Unlike in other patient groups such as aplastic anaemia patients receiving allogeneic transplants it can cause significant neurological symptoms which may be confused with MS relapse. The mechanism of this reactivation is probably related to ATG administration but may be exacerbated by prior immune suppression in this heavily pre-treated group, the majority of whom have received highly active disease modifying therapies in the past. These results demonstrate the importance of monitoring for EBV reactivation following autologous HSCT and the consideration of pre-emptive therapy.

**Disclosure of conflict of interest:** None.

#### P700

##### **Investigation and management of bone mineral density following hematopoietic cell transplant: A survey of current practice by the the complications and quality of life working party of the EBMT**

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Reduced bone mineral density (BMD) is a well recognised complication of HCT. Guidelines recommend scanning by dual energy X-ray absorptiometry (DXA) one year after transplant in all HCT patients<sup>1</sup> or else specific groups of high risk patients.<sup>2,3</sup> It is recognised that both dose and duration of steroids are risk factors for low BMD and it is recommended that prednisolone doses greater than or equal to 5mg/day for more than 3 months should prompt a DXA scan. For patients with osteopenia it is recommended that Calcium/vitamin D supplements are given together with lifestyle advice including diet, smoking cessation and weight bearing exercise.<sup>1</sup> In this survey we have investigated the current practise in investigating and managing bone health in the context of HCT. A survey was sent to all 453 centres including 45 countries registered with EBMT as of November 2016. 63 centres replied from 14 countries. Response numbers to each question were variable and are indicated by the denominators. 5/36 used a national guideline to guide their practise, and 3/34 used an international guideline. No single guideline was quoted more than once. 25/46 (54%) did not conduct routine DXA screens on their patients at any time point. 23 respondents to a question about triggers for DXA screening post transplant gave 74 responses. The most frequent triggers were post-transplant issues (36/74, 48%): steroid use (16/23), vitamin D deficiency (8/23), prolonged immobilisation (5/23), hypogonadism (4/23) calcineurin inhibitor use (3/23). For 19/74 (26%) triggers were pre-transplant issues: family history of osteoporosis (7/23), low BMD pre transplant (5/23), steroids pre HCT (5/23) and low BMI (2/23). Practice of routine surveillance post HCT was indicated by 16/74 (21%) responses as follows: all allografts (8/23), all females >60 years (3/23), all HCT (3/23) or all patients with acute lymphoblastic leukemia. Steroid dose/duration triggers for DXA scanning were variable. The most frequent dose trigger (7/14) was 1 mg/kg given for a minimum duration of 4 weeks (median duration 3 months). Osteopenia was

managed with calcium and vitamin D alone in 28/43 cases (65%) and together with bisphosphonates in 9/43 (21%). Osteoporosis was managed with bisphosphonates ± calcium/vit D in 19/36 and with calcium/vit D alone in 10/36. 8 /34 indicated that they would give bisphosphonates in the absence of osteoporosis, if a patient with osteopenia was receiving long term steroids. Dissemination and implementation of existing guidance on investigation and managing low BMD post HCT appeared to be poor amongst respondents to our survey. Routine DXA scanning was underused; the trigger for DXA in the context of steroids is inappropriately high at many centres at 1 mg/kg daily for 3 months; in established osteoporosis, bisphosphonates were used less frequently than would be anticipated. These findings may reflect the limited data on which current recommendations have been made, or the large number of non-transplant guidelines for investigating and managing low BMD which confound management of this post-HCT patient group.

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**Disclosure of conflict of interest:** None.

#### P701

##### Is autologous hematopoietic stem cell transplantation for acute myeloid leukemia still an option?

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Hematopoietic stem cell transplantation (HSCT) still remains as the most efficient therapy for adult patients with acute myeloid leukemia. For older patients and those lacking a HLA-compatible donor, autologous hematopoietic stem cell transplantation (auto-HSCT) is a valid therapeutic option.<sup>1</sup> Authors aimed for determining the effect of auto-HSCT for acute myeloid leukemia patients and analyze group of patients who underwent auto-HSCT. The study has been set as a retrospective single center study. Clinical information included age, gender, AML type and cytogenetic risk. Pre-transplantation treatment, mobilization and conditioning were analyzed and thus subsequently authors used Kaplan and Meier method to calculate the actuarial overall survival rate. Table 1 describes patients' characteristics. Majority of patients received similar induction therapy based on combination of cytarabine and anthracycline. Timespan from the diagnosis to auto-HSCT varied from 74 days to 1791 days, median was 175 days. Seventy (88,6%) patients received a preparative regimen consisting of busulfan at 1mg/kg orally, four times daily for 4 days for a total dose of 16 mg/kg administered on day -6 through day -3 and melphalan 100–150 mg/m<sup>2</sup> intravenously for over 4 hours on day -2. Patients achieved an absolute neutrophil count (ANC) of  $\geq 0.5 \times 10^9/L$  in between 10 to 40 days; median was 14 days. Patients achieved not transfused platelet count  $\geq 20 \times 10^9/L$  in between 10 to 209 days; median was 19 days. Median of patients' discharge from hospital was 19 days (range: from 13 to 44 days) since auto-HSCT. Hundred day mortality after autologous transplant was at 6.32% (5/79). On the date of our evaluation (April 30, 2016), 48 patients were alive and in continued CR. The relapse rate was 39.5% (32 patients) and 7 patients (8.6%) were lost from follow-up. The 5-year overall survival (OS) was 60.8%, so the target median of overall survival has not been reached.

[P701]

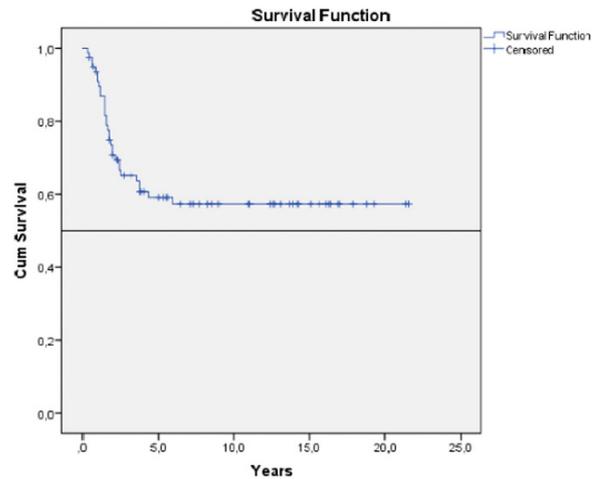
**Table 1. Overall Patient Characteristics**

<b>No. of patients</b>	79	
<b>Age (y)</b>	Range 20-67	Median 44
<b>Sex</b>	Male 44	Female 35
<b>FAB classification</b>	M1-7	M2-35
	M4-20	M5-7
	After MDS 6	Biphenotypic 1
<b>First WBC (<math>\times 10^9/l</math>)</b>	Range 0.78-393	Median 14.75
<b>Risk status</b>	Standard 9	Intermediate 50
	Poor 13	NA 7

Figure 1. Overall survival

The presented clinical study has demonstrated the safety and efficiency of myeloablative chemotherapy followed by auto-HSCT for the treatment of AML and favorably overall survival.

[P701]



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**Disclosure of conflict of interest:** None.

#### P702

##### Lipid profiles after first and subsequent allogeneic and autologous stem cell transplantations: 25-year follow up data

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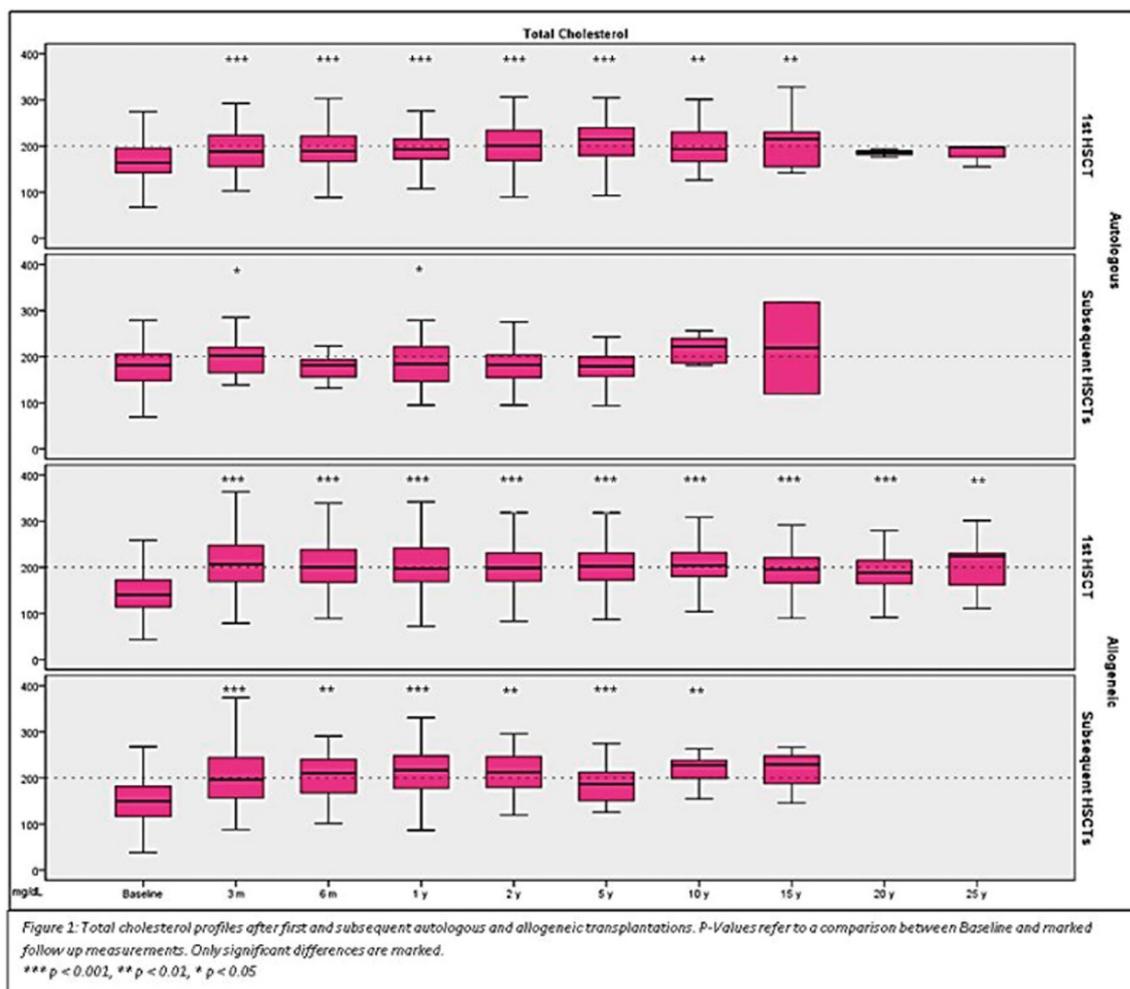
The development of dyslipidaemia is commonly observed after haematopoietic stem cell transplantation (HSCT). Few data are available concerning lipid profiles over a long follow-up period or with regard to the different transplantation types (autologous vs. allogeneic) or the effect of multiple transplantations on the development of dyslipidaemia. A retrospective, single center cohort study including 1239 adult patients (> 16 years) who underwent HSCT at the University Hospital Basel

1973–2013 and who survived  $\geq 100$  days was performed. Patients with at least a baseline lipid measurement were included ( $n = 1096$ ) and grouped according to the type of their first HSCT (autologous or allogeneic). For the examination of the effect of subsequent HSCTs, patients with consecutive transplantations of the same type were included and other patients were censored when a different transplantation type was performed. Serial lipid profiles (total-, LDL- and HDL-

cholesterol and triglycerides) before and after transplantation were examined. Of the 1096 patients, 407 underwent a first, and 89 of these at least one subsequent autologous HSCT. 689 underwent a first, and 85 of these at least one subsequent allogeneic HSCT. Median age of patients at autologous HSCT was 52y (IQR 39–61) and 43y (32–53) at allogeneic HSCT. 62% and 58% were males, median BMI pre-transplant was 25 (22–28) and 24 (22–27). The majority of patients underwent

[P702]

Percentage of patients with dyslipidaemia								
	Baseline	3 m	1 y	5 y	10 y	15 y	20 y	25 y
<b>Autologous 1st HSCT: total patient number</b>	407	260	203	100	52	25	6	3
Patients with total cholesterol $\geq 200$ mg/dl, %*	22	41	40	62	43	64	0	0
Patients with LDL-cholesterol $\geq 140$ mg/dl, %*	22	28	25	44	32	39	0	0
Patients with HDL-cholesterol $\leq 40$ mg/dl, %*	40	46	31	20	25	36	33	67
<b>Allogeneic 1st HSCT: total patient number</b>	689	622	506	314	214	126	76	21
Patients with total cholesterol $\geq 200$ mg/dl, %*	13	54	48	53	56	44	43	69
Patients with LDL-cholesterol $\geq 140$ mg/dl, %*	12	36	33	33	33	24	25	23
Patients with HDL-cholesterol $\leq 40$ mg/dl, %*	62	50	24	20	16	24	23	15



intensive conditioning before HSCT. Median follow-up time was 3.0 years in the autologous and 4.8 years in the allogeneic group, with a maximum follow up time of 26.1 and 34.3 years, respectively. Table 1 shows the number and percentage of patients with dyslipidaemia (1st autologous and allogeneic transplants). The distribution of exact total cholesterol values along with comparisons with baseline measurements according to group are presented in the Figure 1.

\*% based on number of measurements available

Total, LDL- and HDL-cholesterol and TG increased within 3 months of transplantation, regardless whether autologous or allogeneic transplantation or a first or a subsequent transplantation was performed. The percentage of patients with dyslipidaemia accordingly rose significantly within 3 months of transplantation and persisted throughout follow-up. Although patients undergoing an autologous HSCT presented with higher baseline values of total cholesterol, a significantly greater increase post-transplant was observed after allogeneic HSCT. First and subsequent transplantations seem to behave similarly with respect to changes in lipid profiles.

**Disclosure of conflict of interest:** None.

### P703

#### Low pre-transplant testosterone levels in male patients are associated with endothelial vulnerability and non-relapse mortality after allogeneic stem cell transplantation

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Low testosterone has been demonstrated to be an independent determinant of endothelial (dys)function in men. Graft-versus-host disease (GVHD) is a major contributor to non-relapse mortality (NRM) after allogeneic stem cell transplantation (alloSCT). Vulnerability of the recipients' endothelial cell system is a novel concept to explain why a proportion of patients with acute GVHD fail to respond to escalating immunosuppressive therapy and ultimately succumb to GVHD and related complications. This retrospective study investigated the prognostic impact of pre-transplant testosterone levels on NRM after alloSCT in male patients. Between 2002 and 2014, a total of 277 male patients undergoing alloSCT at Heidelberg University (median age 55 years) provided informed consent to participate in this observational study (training cohort). A total of 71 patients (26%) received

transplants from related donors (RD). Diagnoses were AML (48%), MDS (29%), lymphoid malignancies (33%) and multiple myeloma (12%). A total of 176 patients (78%) received statin treatment post alloSCT as per institutional standard policy. For validation, an independent patient cohort of 205 men allografted for AML and MDS (median age 57 years, 18% RD, no statin treatment) at Essen University was analysed. Pre-transplant serum samples were collected between 0 and 2 months before alloSCT and cryopreserved at -80°C. Testosterone and suppressor of tumorigenicity-2 (ST2) levels were measured by radioimmunoassay and ELISA, respectively. Median pre-transplant testosterone level in the training and validation cohort was 13.6 nmol/L (range: 0.3–41.7 nmol/L) and 16.0 nmol/L (0.8–38.1 nmol/L), respectively. In the training cohort, lower pre-transplant testosterone as continuous variable was associated with shorter OS ( $P=0.009$ ). Lower testosterone levels showed a trend towards higher NRM ( $P=0.09$ ) and a significant association with NRM after onset of acute GVHD ( $P=0.02$ ). Multivariate analysis confirmed lower pre-transplant testosterone levels as a significant predictor of an increased NRM risk after GVHD onset ( $P=0.03$ ). In the subgroup of patients not receiving statins post-transplant, lower testosterone levels were associated with increased incidence of transplant-associated microangiopathy ( $P=0.01$ ), and, in addition, with higher pre-transplant ST2 levels indicating endothelial vulnerability. In the validation cohort, similar results with regard to overall survival (OS,  $P=0.02$ ), NRM ( $P=0.04$ ), NRM after acute GVHD onset ( $P=0.03$ ) in univariate analysis, and to NRM after GVHD onset ( $P=0.02$ ) in multivariable analysis could be observed. The association of pre-transplant testosterone levels (in quartiles) and incidence of NRM after GVHD onset in the training and validation cohort is depicted in Figure 1A and 1B, respectively. Our study suggests that low pre-transplant testosterone is associated with serological and clinical evidence for endothelial damage and is an independent risk factor for a fatal outcome of GVHD. Prospective studies in the alloSCT setting investigating testosterone and testosterone supplementation in deficient patients are highly warranted.

**Disclosure of conflict of interest:** None.

### P704

#### NK cells anti-tumor ability in multiple myeloma patients

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[P703]

Percentage of patients with dyslipidaemia								
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Patients with HDL-cholesterol $\leq 40$ mg/dl, %*	40	46	31	20	25	36	33	67
<b>Allogeneic 1st HSCT: total patient number</b>	689	622	506	314	214	126	76	21
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Patients with LDL-cholesterol $\geq 140$ mg/dl, %*	12	36	33	33	33	24	25	23
Patients with HDL-cholesterol $\leq 40$ mg/dl, %*	62	50	24	20	16	24	23	15

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Natural Killer (NK) cells are lymphocytes of the innate immunity with a potent anti-tumor capacity. In tumor patients, such as multiple myeloma (MM) patients, an elevated number of NK cells correlates with a higher overall survival (OS) rate. Our study addressed NK cells characteristics and anti-tumor ability in MM patients. Especially cytotoxicity of patient-derived, cytokine-stimulated NK cells against MM cells has been analyzed at various time points (at diagnosis, before/after chemotherapy and/or auto-SCT). NK cells from patients were analyzed by FACS after PBMCs isolation via Ficoll separation at different time points: TP1, before the start of high dose chemotherapy (HDC)/auto-SCT; TP2, after early 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. 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> NK cell subpopulation (71.86%), after leukocyte recovery at TP2 CD56<sup>+</sup>CD16<sup>-/+</sup> NK cells were the main subsets (CD16<sup>-</sup>: 22.85%; CD16<sup>+</sup>: 36.51%). We further evaluated the NK cell function upon tumor interaction at the defined time points. CD56<sup>+</sup>CD16<sup>-</sup> NK cells were the main subset to produce IFN- $\gamma$  upon interaction with K562 cells at all different time points. The percentage of IFN- $\gamma$ -positive CD56<sup>+</sup>CD16<sup>-</sup> NK cells was slightly decreased at TP2 compared to TP1 but significantly increased from TP2 to TP3 (*P*-value: 0.0008). 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 [*P*-values: 0.0056 (MIP 1 $\beta$ ) and 0.0232 (CD107a)]. Moreover, in a small group of MM patients, 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 compared to NK cells from healthy controls, but the patients NK cells increased their ability to kill MM cells due to the *ex vivo* cytokine expansion. Conclusion: Our data demonstrate that NK cells have an altered phenotype and function after HDC/auto-SCT. Remarkably, these NK cells were able to secrete cytokines and still displayed 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 further improve the patients NK cell activity by an *ex vivo* cytokine stimulation procedure. Finally, we suggest that an additive cell therapy with cytokine-stimulated autologous NK cells might improve the outcome of MM patients.

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**Disclosure of conflict of interest:** None.

#### P705

##### **NKG2D-CAR redirected CD45RA: Memory T-cells target pediatric acute leukemia**

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Lymphoid and myeloid acute leukemia are the most frequent type of cancer and the most frequent cause of cancer related death in children. Relapse and refractory disease are the main clinical problems that current therapies are still unable to solve. 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 leukemia cells and constitute suitable targets for immunotherapy. The expression of NKG2D ligands was analyzed in Peripheral Blood Mononuclear Cells from 61 pediatric patients suffering from acute leukemia (21 Acute Myeloid Leukemia, 25 B cell Acute Lymphoid Leukemia and 15 T cell Acute Lymphoid Leukemia), as well as in 7 leukemia cell lines (K562, RS4-11, Jurkat, NALM-6, MOLT-3, REH and CEM), by flow cytometry using specific monoclonal antibodies directed against MICA, MICB, ULBP-1, ULBP-2, ULBP-3 and ULBP-4, and by quantitative PCR using TaqMan probes. Peripheral blood mononuclear cells from healthy donors were labeled with CD45RA microbeads and depleted using AutoMACS device. The HL20i4r-MNDantiCD19bbz lentiviral vector was 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-HIVgpc, pCAGG-VSVG and pCAG4-RTR2. Cytogenetic studies and array Comparative Genomic Hybridization were performed to analyze the genetic stability of lentiviral-transduced memory T cells. The *in vitro* cytotoxicity of CD45RA<sup>-</sup> T cells against leukemia cells, healthy PBMC and Mesenchymal Stem cells (MSC) was evaluated by performing conventional 4-hour europium-TDA release assays or by flow cytometry using CFSE and 7AAD labeling of target cells. NKG2DL were heterogeneously expressed in leukemia primary cells and cell lines. For B cell ALL primary samples, we found expression of MICA/B, MICA and ULBP1 decreased in refractory disease compared to remission (*P*=0.01, *P*=0.03 and *P*=0.02, respectively). Lentiviral transduction of NKG2D-4-1BB-CD3z markedly increased NKG2D surface expression in CD45RA<sup>-</sup> memory T cells, which became consistently more cytotoxic than untransduced cells against leukemia cells. Additionally, no chromosomal aberrations nor cytotoxic activity against healthy PBMC or Mesenchymal Stem cells was observed in NKG2D CAR expressing T cells. Our results demonstrate NKG2D-CAR redirected CD45RA<sup>-</sup> memory T cells target NKG2DL expressing leukemia cells *in vitro* and could be a promising and safe immunotherapeutic approach for acute leukemia patients.

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**Disclosure of conflict of interest:** None.

#### P706

##### **Optimal initial dose of busulfan in infant and child: Comparison of busulfan pharmacokinetics among age groups**

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Busulfan is one of essential drugs for hematopoietic stem cell transplantation (HSCT). Because of its narrow therapeutic range: targeted busulfan using therapeutic drug monitoring (TDM) has been used. Generally, the initial dose of busulfan is determined by patients' body surface area as 120 mg/m<sup>2</sup> except for infants (80 mg/m<sup>2</sup>). However, pharmacokinetic evidence of these initial doses is scarce. Therefore, we investigated the full pharmacokinetics of busulfan in infant and child, and attempted to validate that these initial doses are acceptable. One hundred ninety-five pediatric patients undergoing HSCT using four-day targeted busulfan were enrolled. Of them, 6 patients received HSCT when their age was ≤ 1 year old (infant group [IG]), and 19 patients received when 1–2 years old (toddler group [TG]). The remaining 170 patients were defined as a child group (CG). Busulfan was administered intravenously once daily for 4 consecutive days. TG and CG received 120 mg/m<sup>2</sup> as the first dose, and IG received 80 mg/m<sup>2</sup>. Using daily TDM, we adjusted the next dose of busulfan. Target daily and total area under the curve (AUC) were 18 750 µg·h/L/day and 74 000–76 000 µg × h/L, respectively. Median first-day busulfan AUC of IG, TG, and CG were 18 416, 22 529 and 20 410 µg × h/L, respectively, which was significantly different ( $P=0.031$ ). However, there was no significant difference in median total busulfan AUC (IG; 74 180, TG; 73 406, and CG; 74 482 µg × h/L, respectively,  $P=0.089$ ). The coefficient of variance (CV) of four-day busulfan AUCs in IG and CG was similar (median CV: 22.1% and 24.7%, respectively), whereas CV of TG was 40.4%. In sub-analysis of TG and CG who received equally 120 mg/m<sup>2</sup> as the first dose, there was an inverse correlation between age and first-day busulfan AUC ( $r=-0.148$ ,  $P=0.042$ ), as well as between age and CV of four-day busulfan AUCs ( $r=-0.210$ ,  $P=0.004$ ). Initial busulfan dose as 80 mg/m<sup>2</sup> for infant could be acceptable in aspect of first-day AUC and CV of four-day busulfan AUCs. However, higher first-day AUC and CV were shown in TG. Although target total busulfan AUC could be achieved safely by TDM, we suggest that reduction of initial dose less than 120 mg/m<sup>2</sup> is also necessary to patients with 1–2 years old to lower the relatively higher first-day AUC. Taken together, TDM is highly recommended to reduce busulfan toxicity, especially in younger children.

**Disclosure of conflict of interest:** None.

#### P707

##### Outcome and future perspectives of allogeneic stem cell transplantation in patients with acute myeloid leukemia according to ELN risk

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Post-induction treatment strategy of acute myeloid leukemia (AML) is currently driven by European Leukemia Net (ELN) risk assessment at diagnosis. If it is well established that patients belonging to favourable-risk group can be treated with chemotherapy and/or autologous stem cell transplantation (SCT) and that those belonging to the unfavourable-risk group should be addressed to allogeneic (allo) SCT, for patients included in the intermediate-risk groups the best post-induction treatment has not been established yet. We report here a 6-years (2010–2015) allo-SCT single Center experience in 78 AML patients. Median age was 53 years (range: 20–68), 17%, 23%, 9% and 51% were grouped in the ELN favourable, intermediate-I, intermediate-II and unfavourable risk category, respectively and 47% of the patients were allografted in advanced disease-phase (2nd complete remission). Half of the patients received a sibling HLA compatible donor, 76% of the cases

received peripheral blood stem cells and half of the patients received a myeloablative conditioning regimen. Graft versus host disease prophylaxis was conventionally based on cyclosporine and short-course methotrexate, with the addition of anti-lymphocyte immunoglobulin in case of matched unrelated donor. The clinical and transplant characteristics of the patients according to the ELN-risk group were well balanced. With a median follow up of 20 months (range: 8–58 months), the projected 2 years overall survival (OS) and disease free survival (DFS) is 45% (95% CI: 32–57%) and 43% (95% CI: 30–54%). The median OS and DFS in favourable/intermediate-I vs intermediate-II/unfavourable is 21.8 and 14.8 months (Figure 1A;  $P=0.67$ ) vs 18 and 14.8 months (Figure 1B;  $P=0.66$ ). The relapse rate (RR) and the non relapse mortality (NRM) at two years are 38% (95% CI: 26–50%), and 15% (95% CI: 8–26%), respectively. Non differences were observed comparing the 2 years RR and the 2 years NRM of patients in the favourable/intermediate-I vs intermediate-II/unfavourable ELN risk group (36% vs 43%;  $P=0.66$  and 16% vs 18%;  $P=0.95$ ). Interestingly, the percentage of patients allografted in advanced phase of the disease was higher in those included in low/intermediate-I with respect to intermediate-II/unfavourable ELN-risk group (73% vs 43%;  $P=0.001$ ). Our data suggest that allo-SCT can cure approximately 40–50% of AML patients, with no difference within the ELN risk groups. Disease recurrence remains the major problem and this is highly correlated to the percentage of patients in advanced phase of the disease at transplant, particularly in ELN favourable/intermediate-I patients. We are currently collecting the data on minimal residual disease (MRD) status of these patients during chemotherapy and before transplant using molecular biology on target genes and/or multiparametric flow cytometry on leukemia associated immunophenotype, in order to assess if the prognosis of these patients may be refined by the prospective application of MRD data.

[P707]

Figure 1A. Overall survival of the 78 AML patients according to ELN-risk group

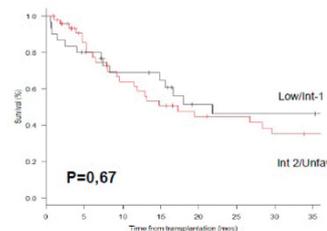
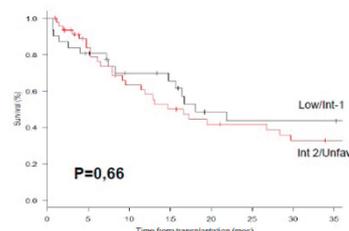


Figure 1B. Disease free survival (DFS) of the 78 AML patients according to ELN-risk group



**Disclosure of conflict of interest:** None.

#### P708

##### Outcome of allogeneic stem cell transplantation for patients with high-risk acute leukemia according to donor type and graft-versus-host disease prophylaxis

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In high-risk acute leukemia (HR-AL), allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment. Increasingly, HSCT is being performed utilizing alternative donors. We retrospectively analyzed the outcome of 148 consecutive patients (pts) with HR-AL (AML/ALL,  $n = 118/30$ ) undergoing first allogeneic HSCT in our transplant unit between 1/2011 and 6/2015 according to donor type and graft-versus-host disease (GVHD) prophylaxis: In the matched related donor group (MRD,  $n = 26$ ), HSCT was performed with standard immunosuppression (IS), that is, calcineurin inhibitor (CNI) plus methotrexate or mycophenolate mofetil (MMF). For 10/10 HLA-allele matched unrelated donors (10/10 MUD,  $n = 84$ ) or 9/10 HLA-allele MUD (9/10 MUD,  $n = 24$ ) we used IS and anti-thymocyte globulin (ATG Fresenius/Neovii). HSCT with a haploidentical family donor or an 8/10 HLA-allele mismatched unrelated donor was performed using IS with CNI plus MMF and post-transplant cyclophosphamide (PT-Cy,  $n = 14$ ). A myeloablative ( $n = 75$ ) or reduced-intensity ( $n = 73$ ) conditioning regimen was applied in complete remission (CR,  $n = 106$ ) or active disease ( $n = 42$ ). Pts had a median age of 52 years (range: 19–72) and hematopoietic cell transplantation comorbidity index of 3 (range: 0–11). Patient and treatment characteristics were well balanced between the groups except for a higher percentage of pts transplanted in CR in the PT-Cy group (93% vs. 54–76%,  $P = 0.03$ ). Peripheral blood stem cells were preferred for MRD, 10/10 MUD and 9/10 MUD (81%, 95% and 96%, respectively) and bone marrow for 93% of PT-Cy based HSCT. All pts engrafted. With a median follow-up of 28 months (range: 1–60), probability of overall survival (OS) at 3 years was  $54 \pm 10\%$  for the MRD,  $65 \pm 6\%$  for the 10/10 MUD,  $41 \pm 11\%$  for the 9/10 MUD and  $93 \pm 7\%$  for the PT-Cy group, without significant differences ( $P = 0.05$ ). However, the probability of achieving the combined endpoint GVHD- and relapse-free survival (GRFS) at 3 years varied significantly between the groups (MRD  $8 \pm 5\%$ , 10/10 MUD  $43 \pm 6\%$ , 9/10 MUD  $29 \pm 10\%$  and PT-Cy  $57 \pm 13\%$ ,  $P < 0.01$ ), reflecting the high cumulative incidence (CI) of chronic moderate and severe GVHD at 1 year in the MRD ( $58 \pm 11\%$ ) as opposed to the other groups (10/10 MUD  $12 \pm 4\%$ , 9/10 MUD  $12 \pm 8\%$  and PT-Cy  $33 \pm 14\%$ ,  $P < 0.01$ ). Of note, donor type had no impact on CI of transplant-related mortality (TRM) at 3 years ( $12 \pm 3\%$ ), acute GVHD G3-4 at day +100 ( $10 \pm 3\%$ ) or leukemic relapse at 3 years ( $34 \pm 4\%$ ). Overall, AML pts  $>60$  years of age had a significantly inferior relapse-free survival compared to younger pts ( $50 \pm 9\%$  vs.  $71 \pm 6\%$ , respectively,  $P < 0.01$ ) without a

higher CI of TRM ( $P = 0.37$ ). Median time to AML relapse was 6 months. Our results suggest that PT-Cy-based alternative donor HSCT is safe in HR-AL pts and provides a solid basis for a randomized clinical trial comparing HSCT from haploidentical family donors and 9/10 MUD, currently in preparation. While OS did not vary between groups, GRFS was dismal after MRD transplants without ATG, due to high rates of severe chronic GVHD, consistent with published data. As leukemic relapse remains the major cause for treatment failure especially in elderly pts, maintenance strategies using novel drugs or cellular therapies are warranted.

**Disclosure of conflict of interest:** None.

**P709**

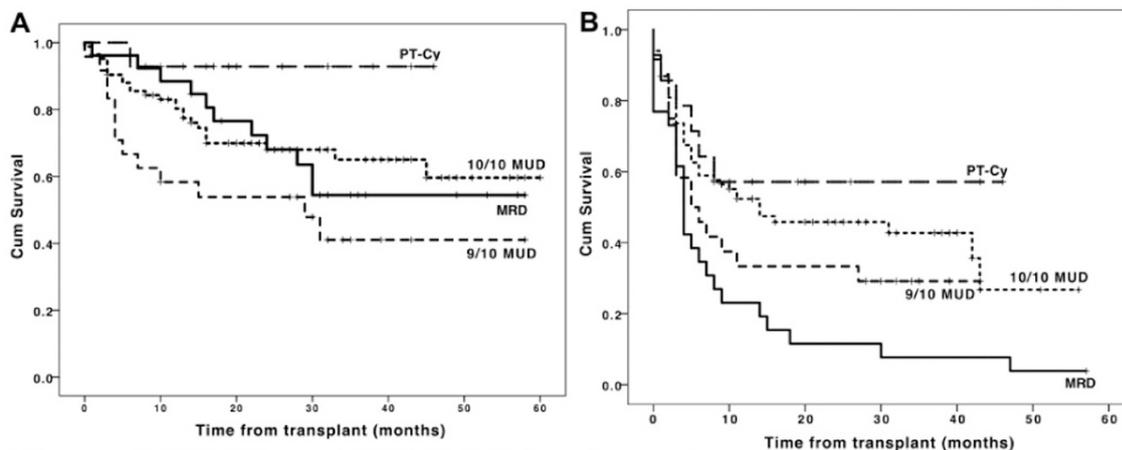
**Outcome of second allogeneic hematopoietic stem cell transplant in children with malignant and non-malignant underlying diseases. A Single center retrospective analysis**

I Zaidman and I Zaidman<sup>1,2,3,4,5,6,7</sup>

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Relapse following hematopoietic stem cell transplant (HSCT) is the leading indication for a second transplant in patients with malignant disease. HSCT has been shown to be superior to chemotherapy alone or palliative measures in these patients. For non-malignant disease a second transplant may be considered for graft failure after first transplant. Data regarding the outcome of a second HSCT for non-malignant disease is scarce. We retrospectively analyzed 29 patients who underwent a second HSCT, for survival and toxicity data. Twenty-nine patients (age 0–19 years) who received a second HSCT at our institution during 1998–2015 were included in the analysis. Thirteen patients had an underlying malignancy and 16 patients were transplanted for non-malignant indications, including inborn errors of metabolism, non-malignant hematologic diseases and immune deficiency. Median follow up was 14 months (range: 1–180). There were 10 deaths (77%) in the malignant group, 7 (53%) were due to disease relapse and 3 (23%) were transplant related. Fifty percent of deaths occurred within the first year following the second HSCT. In the non-malignant group there were 5 deaths (31%), of which 2 (12%) were attributed to the underlying disease and 3 (18%) were transplant related. All deaths but one occurred within the first year post HSCT. Treatment related mortality following second HSCT is higher compared to first transplant. The higher survival rate in the non-malignant group suggests that transplant following graft failure should be considered in

[P708]



**Figure 1 OS (A) and GRFS (B) by donor type**

patients with otherwise incurable underlying disease. Though the outcome for patients with relapse of malignant disease following HSCT is poor, a second transplant may benefit a subset of these patients. Attempts to achieve complete remission prior to transplant should be made to improve outcome. Due to the small number of patients in our cohort, further multi-center trials are needed.

**Disclosure of conflict of interest:** None.

#### P710

##### **Outcomes of hematopoietic stem cell transplantation in severe combined immunodeficiency patients with disseminated bacillus Calmette Guerin infection**

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Disseminated BCG infection (BCG-osis) is a rare but most serious complication in vaccinated especially immunocompromised children. Severe Combined Immunodeficiency Disorder (SCID) is probably the commonest primary immunodeficiency associated with BCG-osis, though there is no such definitive data as most of the cases described in literature are in the form of reports. Hematopoietic Stem Cell Transplantation (HSCT) is a life-saving treatment for patients with SCID, especially if therapy is instituted early, prior to onset of infections. As BCG vaccine is routinely given to all Iranian children at birth, the likelihood of having an active infection at the time of transplant would be significantly high. The main objective of this study was to evaluate the outcomes of HSCT in SCID patients with disseminated BCG infection. Sixteen SCID patients underwent HSCT in our center since 2007 to 2016, of which nine patients (7 male, 2 female) were enrolled in this analysis. All the 9 patients had received BCG vaccination according to the national vaccination protocol, and had undergone anti-tuberculosis (TB) treatment prior to transplant due to disseminated BCG infection. The mean age at HSCT was 9.3 months (range: 6–13 months). Patients received bone marrow ( $n=1$ ), peripheral blood progenitor cells ( $n=6$ ) or umbilical cord blood grafts ( $n=2$ ) from HLA-matched related donors ( $n=7$ ) and mismatched unrelated donors ( $n=2$ ). Three patients received unconditioned matched sibling donor transplants and RIC regimen was provided with Fludarabine, Melphalan and Rabbit anti-thymocyte immunoglobulin (Thymoglobulin) in others. Cyclosporine A and Prednisolone were used as graft-versus-host disease (GvHD) prophylaxis. They also continued to receive anti-TB treatment. All patients but one engrafted. The median times to neutrophil and platelet engraftments were 12 days (range: 11–39), and 18 days (range: 17–90), respectively. Engraftment with full chimerism ( $>95\%$ ) occurred in 5 patients and the other 3 patients had mixed chimerism. With a median follow-up of 24 months (range: 3–48 months), overall survival was 66.7%. The main cause of death was disseminated BCG infection. Three out of 8 patients who achieved engraftment, developed acute GvHD (grade I–II), while one patient developed extensive chronic GvHD. Although anti-TB treatment continued, Tuberculous dactylitis occurred in 3 patients post-HSCT that were successfully treated. On last post-HSCT follow-up, 4 patients with full chimerism and 2 with mixed chimerism are alive and disease free. SCID is called as a pediatric emergency as it invariably leads to fatality in infancy without early aggressive therapy and HSCT. In HSCT recipients, the impaired cellular immunity renders these patients more susceptible to infection. As previous reports suggest, our study demonstrates that with appropriate anti-TB cover, immunological reconstitution with complete recovery from BCG infection can be achieved by early HSCT.

**Disclosure of conflict of interest:** None.

#### P711

##### **Paraproteinemia occurrence after allogeneic hematopoietic stem cell transplant as a possible marker for chronic GvHD onset**

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Transient monoclonal gammopathy is commonly reported after solid organ or stem cells transplant (SCT) for hematologic malignancies. However the clinical significance of a paraproteinemia appearance is not fully understood, because the attempts to correlate its effect on survival rates, graft versus host disease (GvHD) occurrence and viral reactivations have led to controversial results. Starting from these reports we decided to evaluate among our allogeneic transplanted patients the incidence of M-component and its possible relationship with chronic GvHD. One-hundred and one patients undergoing alloSCT at the Hematology Unit of Alessandria (Italy) between 2006 and 2015 were evaluated. 55% of patients were male and 45% were females. Pretransplantation diagnosis included: 62 acute myeloid leukaemia/high-risk myelodysplastic syndromes (62%), 14 acute lymphoblastic leukaemia (14%), 13 lymphoproliferative disorders (13%) and 12 other less common malignancies (12%). Patients with multiple myeloma were excluded from the study. All patients had, at least, two pre-transplantation serum electrophoresis with no evidence of pre-existing monoclonal component. Serum electrophoresis was scheduled to be performed at 90, 180 and 360 days and 2 years after transplantation. Forty-nine patients were submitted to alloSCT from a sibling donor and 52 from a matched related donor (MUD); *in vivo* T-cell depletion with anti-thymocyte globulin was used in 63 patients. Thirty-four patients relapsed after alloSCT, 52 (52%) developed chronic GvHD and 56 patients (56%) are currently alive at the last follow-up. Post-transplantation follow up ranged from 81 to 2695 days with a median of 496 days. Paraproteins were detected in 52 out of 101 patients (52%), being monoclonal in 28 patients, and bi or tri-clonal in the remaining cases; the immunoglobulin subclass most commonly observed was IgG. Ten-year overall survival of the whole population was 50%; splitting the population in two cohorts (with or without paraproteinemia) we did not detect any statistical differences in overall survival, GvHD development and relapse incidence at +90 and +180 days post-transplant; viceversa, after 360 days, a statistically significant difference was observed in chronic GvHD occurrence in patients with or without paraproteinemia (85% vs 42%, respectively,  $P < 0.001$ ). Ten-year overall survival curves were significantly better in patients with paraproteinemia as compared with the paraprotein-free group (59% vs 45%,  $P=0.04$ ), and an even more evident significance was seen in ten-year relapse free survival curves (66% for patients with paraprotein vs 48% for patients without paraprotein,  $P=0.009$ ). Monoclonal gammopathy, also in our experience, is frequent following allo-SCT. We observed a strong correlation between the occurrence of paraproteinemia, chronic GvHD and a significantly better overall and relapse-free survival. Recently many evidences showed that B cells are involved in the pathogenesis of chronic GvHD (cGVHD) and anti-B-cell therapy has been suggested for the treatment of cGVHD. We speculate that the presence of a monoclonal gammopathy after allogeneic transplant is expression of the activation of the B-cell compartment. A prospective study with a larger population should be considered, in order to confirm our results and assay post-transplantation monoclonal gammopathy as an early marker for GvHD development.

**Disclosure of conflict of interest:** None.

**P712**

**Peripheral blood stem cell mobilization and collection from elderly patients (≥65 years) with multiple myeloma: A single center experience**

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High-dose melphalan followed by autologous hematopoietic cell transplantation (auto HSCT) has become the standard procedure for patients with symptomatic multiple myeloma (MM). The ability to mobilize stem cells from healthy donors shows little deterioration with age, the influence of patients' age on auto HSCT is uncertain and studies in patients' ≥ 65 years are scarce. Severe studies specific to MM have failed to show an independent effect of patient age on CD34+ mobilization. We retrospectively compared myeloma patients below the age of 65 with patients above 65 years of age, analyzing CD34 mobilization into peripheral blood and the number of leukapheresis needed to collect at least one single stem cell graft. Material and Methods: From February 1999 through April 2016, data from 501 myeloma patients below the age of 65 were compared to 52 myeloma patients above 65 years of age. All these data were obtained from the Ankara University Faculty of Medicine Center for Therapeutic Apheresis and written informed consent was signed according to our institution regulations. Most of the patients received only G-CSF at a dose of 5 µg/kg BW twice-daily s.c. until stem cell procurement. Patients underwent further PBSC collections until we obtained the target dose > 20 CD34+ cells/µL blood. A maximum of 3 collections were performed in the first mobilization; if the cell dose was not achieved, we submitted patients to a second mobilization. Fifty two of 553 patients were above 65 years of age (median age 66, range: 65–73) and 501 patients were below the age of 65 (median age 54, range: 29–64). Baseline characteristics of the older and younger patient cohorts are summarized in Table 1. Mobilization regimens for the younger patient population were cyclophosphamide based (n: 122), G-CSF only (n: 372) and +plerixafor (n: 7). Mobilization in the older population was with cyclophosphamide based (n: 10), G-CSF only (n: 41) and +plerixafor (n=1). The chemotherapy regimens were not statistically different between both age groups. There were no significant statistical differences in time from diagnosis to mobilization, number of prior therapies or disease status between both

[P713]

**Table 1**

Serology	OS*	DFS*	AL relapse*	NRM*	Acute GvHD**	Chronic GvHD*
D-CMV-/R-CMV- (n=23)	12%(0;32)	13%(0;33)	28%(11;48)	60% (34;78)	35%(16;54)	6%(0;24)
D-CMV+/R-CMV- (n=40)	24%(10;38)	25%(11;39)	28%(15;43)	47%(30;62)	35%(21;50)	10%(2;23)
D-CMV-/R-CMV+ (n= 16)	28%(3; 53)	31%(6;56)	20%(4;44)	49%(20;72)	19%(4;41)	32%(9;60)
D-CMV+/R-CMV+ (n= 84)	31%(20;42)	27%(17;37)	27%(18;38)	45%(34;56)	37%(27;47)	10%(4;20)

\*Probability at 8 years (95%CI). \*\*Probability at 100 days (95%CI)

patient groups. The number of CD34+ circulating cells before scheduled leukapheresis was mean 69.28 cells/µL (median 49 cells/ µL, range: 2–397; SEM ± 46.875) in all patients (including patients who failed mobilization). Our data support the observation that after a standard mobilization regimen with anti-myeloma chemotherapy and once-daily growth factor support, patients above 65 years of age show an impaired CD34 mobilization into peripheral blood compared to a younger population. This can be overcome by an increased number of leukaphereses. Still the number of progenitor cells in the actual graft is inferior compared to the younger population.

[P712]

Patients	≥65 years	<65 years	p<0.05
Gender (M/F) (n)	42/10	247/155	
Median age (years (range))	66.5 (65-75)	54 (15-64)	0.00*
Mobilization Regimens(n)			
G-CSF	41	372	
Chemotherapy	10	98	
Plerixafor	1	7	
Median number of leukapheresis (range)	2 (1-3)	2 (1-3)	0.56
Peripheral CD34 (+) cells/µL (first mobilization attempt)	53.56±42.76	86.73±67.77	0.03*
Mean CD34 (+) cells/ml (x10 <sup>6</sup> /kg) (first mobilization attempt)	4.30±2.99	6.92±1.92	0.01*

**Disclosure of conflict of interest:** None.

**P713**

**Prognostic impact of cytomegalovirus serology in donors and recipients of allogeneic hematopoietic stem cell transplantation for acute leukemia. Single center study**

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Donor and/or recipient cytomegalovirus (CMV) seropositivity has been associated with a poor overall survival (OS) in patients who have received an allogeneic hematopoietic stem cell transplantation (alloHSCT). In comparison with seronegative donors, HSCT from seropositive donors has been associated with decreased disease-free survival (DFS) and increased non-relapse-related mortality (NRM). We analyzed the prognostic impact of CMV serology status (donor/recipient) in patients diagnosed with acute leukemia (AL)

who had received an alloHSCT in our institution. Retrospective unicentric study of patients diagnosed with AL between 2001 and 2015 who received alloHSCT. The following outcomes were studied: OS, DFS, and cumulative incidences of relapse (RI), NRM, acute graft-versus host disease (aGvHD) and chronic GvHD (cGvHD). The series included 163 patients (86 males, 77 females), median age of 44 years [15–69]. AL type: 42 (26%) ALL, 121 (74%) AML. Type of transplant: 88 (54%) related donor, 42 (26%) unrelated donor and 33 (20%) unrelated umbilical cord blood. The majority, 111 (68%), received myeloablative conditioning. Stem cells source: peripheral blood 124 (76%), cord blood 33 (20%) and bone marrow 6 (4%). CMV serology status: positive receptor 124 (76%), negative receptor 39 cases (24%); Positive donor 100 (61%), negative donor 63 (39%). Serology status combinations (D/R): +/+ 84 (52%), +/- 40 (24%), -/- 23 (14%), -/+ 16 (10%). 56 patients developed aGvHD and 14 (9%) cGvHD. The impact of donor/recipient CMV serology status on OS, DFS, RI, NRM and incidence of aGvHD and cGvHD for the overall series is reported in Table 1. No statistically significant differences were detected in any of the analyzed variables. In this study, donor/recipient CMV serology showed no influence on the analyzed variables OS, DFS, AL relapse, NRM, acute and chronic GvHD. However, the sample size limits the validity of the results.

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#### P714

##### **Quality of life (QoL) assessment in multiple sclerosis (MS) patients undergoing autologous hematopoietic stem cell transplantation (AHST): does it matter?**

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During the last two decades AHST has been used as a treatment option for MS with promising outcomes. QoL is an important outcome of MS treatment. Its assessment gives the patient's perspective on the overall effect of treatment. We aimed to study QoL in MS patients before and after AHST and search the value of the data obtained for decision-making. A total of 135 patients with different types of MS were enrolled in the study: mean age—34 (range—17–54) years old; male/female—53/82; mean EDSS—3.5 (range: 1.5–8.5). All patients were treated by AHST. Reduced-intensity BEAM-like conditioning was used (BCNU 300 mg/m<sup>2</sup>, etoposide 100 mg/m<sup>2</sup>, Ara-C 100 mg/m<sup>2</sup> and melphalan 100 mg/m<sup>2</sup>). Mean follow-up was 24 months (range: 12–53 months). QoL was assessed using generic questionnaire SF-36. For comparisons t-test for independent samples or Mann–Whitney test was used. QoL parameters in MS patients at 12 months after AHST improved in comparison to base-line: physical functioning—66.3 vs 52.6, role-physical functioning—62.8 vs 43.8, bodily pain—78.2 vs 76.4, general health—64.1 vs 56.7, vitality—62.8 vs 45.4, social functioning—72.4 vs 57.7, role-emotional functioning—68.0 vs 55.6, and mental health—72.1 vs 58.6. Further QoL improvement was registered at long-term follow-up: Integral QoL Index exhibited 0.50 at long-term follow-up as compared to 0.32 at base-line. QoL improvement was more dramatic in relapsing-remitting MS than in progressive MS. We found a significant increase of all eight SF-36 scales in a year post-transplant as compared with base-line in relapsing-remitting MS patients ( $P < 0.05$ ). In progressive MS patients statistically significant improvement was registered for six out of eight SF-36 scales (except bodily pain and role-emotional functioning) ( $P < 0.05$ ). Improved QoL parameters were preserved over the entire study period in all the patients who did not have disease progression or relapse. In conclusion, QoL monitoring

in MS patients after AHST provides clinicians with the unique information regarding the changes in physical, psychological and social well-being of patients who have been treated with this new treatment modality. It allows to evaluate risks/benefits of MS patients undergoing AHST and might influence decision-making. Further studies are needed to examine the trajectory of QoL changes in this patient population to better define treatment outcomes after AHST.

**Disclosure of conflict of interest:** None.

#### P715

##### **Reduced-intensity conditioning hematopoietic stem cell transplantation in leukocyte adhesion deficiency type I: A single center experience**

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Pediatric patients with leukocyte adhesion deficiency type-I (LAD-I), a rare autosomal recessive primary immunodeficiency disorder, experience severe and recurrent lifethreatening bacterial infections. Allogeneic haematopoietic stem cell transplantation (HSCT) offers the possibility of curative therapy although the conditioning regimen used for HSCT in LAD-I is still a controversial issue. This study provides evaluation of outcome of the LAD-I pediatric patients who underwent Reduced-Intensity Conditioning (RIC) HSCT. Twenty four patients (14 female) with severe LAD-I who received 26 HSCTs between February 2007 and September 2016 at our center were enrolled. The median age at HSCT was 30 months (range: 4 months–14 years). Patients received bone marrow ( $n=9$ ), peripheral blood progenitor cells ( $n=14$ ) or umbilical cord blood grafts ( $n=3$ ) from HLA-matched related donors ( $n=18$ ), mismatched related or unrelated donors ( $n=4$ ), unrelated fully matched donors ( $n=1$ ) and haploidentical relative donors ( $n=1$ ). RIC regimen was provided with Fludarabine, Melphalan and anti-thymocyte immunoglobulin. Cyclosporine A and Prednisolon were used as graft-versus-host disease (GvHD) prophylaxis. Engraftment occurred in 23/26, of which one patient experienced graft rejection. The median times to neutrophil and platelet engraftments were 12 days (range: 10–23 days) and 15 days (range: 10–32 days), respectively. With a median follow-up of 43 months (range: 2–95 months), overall survival (OS) was 70.8%. The main causes of death were GvHD and infection. Acute GvHD occurred in ten patients (4 grade I–II, 6 grade III–IV) and 3 patients also developed chronic GvHD. There were no significant differences in acute GvHD occurrence and also OS regarding to the stem cell sources. At this time, 10 patients with full chimerism and 6 patients with mixed chimerism are alive and disease free. Conclusion: HSCT offers long term benefit in LAD-1 and should be considered as an early therapeutic option if a suitable HLA-matched stem cell donation is available. As pre-transplant infections in primary immunodeficient patients especially those affected by LAD-1 lead to rise in mortality rate, RIC regimen is found to be safe and mixed donor chimerism appears sufficient to prevent significant symptoms.

**Disclosure of conflict of interest:** None.

#### P716

##### **Regulatory T cells (TREGs) GMP production for clinical application: Phenotypic and functional analysis of cryopreserved/thawed healthy TREGs**

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TREGs based immunotherapy may be beneficial in several immune mediated diseases including Graft Versus Host Disease (GVHD). The possibility of cryopreserving TREGs might lead to the administration of multiple doses, thus potentially increasing their efficacy in chronic diseases. However, there are few and controversial data on the functionality of TREGs after cryopreservation. Here, we evaluated the phenotype and the inhibitory capacity of thawed TREGs. TREGs were purified from leukapheresis of normal donors ( $N=3$ ) by double immunomagnetic depletion (CD8 and CD19) followed by CD25 enrichment using the CliniMACS system (Miltenyi Biotec) under GMP condition. The cells were cryopreserved in saline solution containing 10% Human Serum Albumin (HSA) and 10% DMSO with a controlled-rate freezing. Cell viability was assessed by 7-AAD staining. Number/phenotype and function were evaluated on fresh and thawed TREGs. Cryopreserved autologous T effector (Teff) cells were used in MLR assays. Before cryopreservation the TREGs enriched product mean viability was  $95 \pm 4\%$  and the mean percentage of CD45+CD4+CD25+CD127low and CD45+CD4+CD25+CD127lowFoxP3+ cells was  $74 \pm 13\%$  and  $66 \pm 10\%$ , respectively. We then analysed the TREGs enriched product after thawing. Mean viability of thawed TREGs, by 7-AAD staining, was  $85 \pm 7\%$ . The viable TREGs were almost totally CD4+CD25+ ( $97 \pm 2\%$ ). The mean percentage of CD4+CD25+CD127low and CD4+CD25+CD127lowFoxP3+ thawed cells was  $73 \pm 14\%$  and  $71 \pm 20\%$  respectively. The contaminant cells present in the TREG enriched product were mostly CD4+CD25+CD127+ (around 18%). We further characterized the phenotype of the CD4+CD25+CD127low population. This population was almost totally Foxp3+ ( $93 \pm 6\%$ ) and expressed selected markers at various degree (CD62L ( $50 \pm 2\%$ ), CD15s ( $6 \pm 2\%$ ), CD45RA+ ( $19 \pm 3\%$ ), HLA-DR+ ( $15 \pm 10\%$ ), CCR7+ ( $74 \pm 5\%$ ), CD49d ( $52 \pm 14\%$ ), CD26+ ( $1 \pm 0.4\%$ ), CD196+CD161+ ( $4 \pm 1\%$ ). Notably, viable thawed TREGs were able to induce inhibition of autologous Teff cells in a 1:2 Tregs:Teff ratio as freshly isolated TREGs:  $44 \pm 16\%$  (thawed) vs  $55 \pm 24\%$  (fresh) of inhibitor ( $P > 0.1$ ). In conclusion, here we demonstrated that thawed TREGs from healthy donors maintain a stable phenotype. In addition, in our hands TREGs show good suppressive ability after thawing despite lower expression of CD62L and CD15s (markers of most suppressive TREGs) as compared with the available published data (Florek et al 2015; Miyara et al 2015).

**Disclosure of conflict of interest:** None.

**P717**  
**Relapsed and refractory malignant B cell diseases: Evidence for therapeutic efficacy via subcutaneous administration of anti-CD20 x anti-CD3 antibody lymphomun**

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The trifunctional antibody anti-CD20 x anti-CD3 lymphomun represents a chimeric immunoglobulin scaffold (mouse IgG2a/ rat IgG2b) with promising treatment outcome in patients suffering from malignant B cell diseases. By changing the lymphomun administration route from intravenous (i.v.) to subcutaneous (s.c.) the proinflammatory cytokine-mediated side effects were considerably slighter and generally well-tolerated. Most importantly, s.c. lymphomun showed outstanding responses in B cell depletion even in the absence of elevated cytokine levels (e.g. IL-6) that are required for cytotoxic T cell activation. In summary, the clinical tolerability of s.c. lymphomun may result in a considerable improvement of the subjective well-being and in enhanced mobility due to decreased pain symptomatology.

**Disclosure of conflict of interest:** Horst Lindhofer is the CEO of Trion Research and the inventor or co-inventor of several trifunctional antibody patents. Peter Ruf and Juergen Hess are employees of Trion Research. Raymund Buhmann and Martin Dreyling are co-inventors of a trifunctional antibody patent. The other authors disclose no potential conflicts of interest.

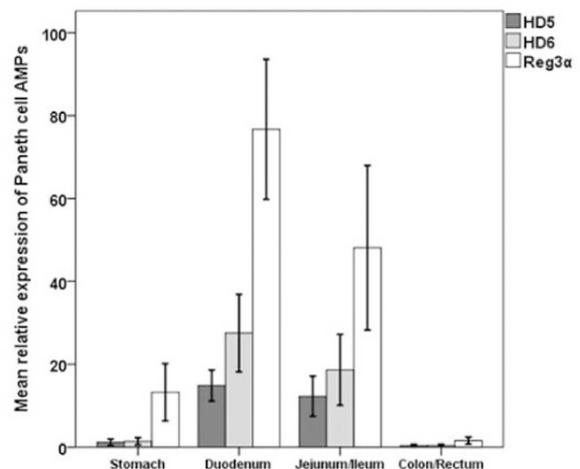
**P718**  
**Release of antimicrobial peptides in allogeneic stem cell transplantation: Opposing effect of graft-versus-host disease and systemic antibiotics**

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Intestinal microbiota disruption is associated with acute gastrointestinal (GI) GvHD and inferior outcome in patients after allogeneic stem cell transplantation (ASCT). The wide use of systemic broad spectrum antibiotics adds a further risk factor contributing to major microbiota shifts. Here, in a retrospective analysis of 200 patients undergoing ASCT at the Regensburg University Medical Center we assessed the relative expression of Paneth cell antimicrobial peptides (AMPs) in 292 human intestinal biopsies in relation to acute GI GvHD and systemic antibiotic treatment. The relative expression of Paneth cell AMPs was significantly higher in biopsies of the upper GI tract than in the lower GI tract for Reg3a ( $P \leq 0.001$ ), human defensin (HD) 5 ( $P \leq 0.001$ ) and HD6 ( $P \leq 0.001$ ). Regarding the distribution of Paneth cell AMPs in the GI tract we observed significantly higher expressions of all three Paneth cell AMPs in the duodenum, jejunum and ileum compared to the stomach, colon and rectum ( $P < 0.001$ , Figure 1). In the presence of acute GI GvHD, Paneth cell AMPs reacted contrarily in the upper and lower GI tract: We observed a decrease of HD5, HD6 and Reg3a in the upper GI tract ( $P \leq 0.01$ ), similarly Paneth cell count dropped in case of severe GI GvHD stage 2-4 ( $P < 0.001$ ). However in the lower GI tract severe acute GI GvHD was associated with an increase of Paneth cell AMPs ( $P \leq 0.03$ ). Initiation of additional systemic antibiotic treatment prior to day 10 after ASCT correlated with a significantly higher expression of HD5 ( $P = 0.002$ ) and Reg3a ( $P = 0.01$ ) in intestinal biopsies compared to patients without or with initiation of systemic antibiotic after day 10. However, no significant differences were found in terms of HD6 expression in intestinal biopsies and start of systemic antibiotic therapy. The expressions of HD5, HD6 and Reg3a in intestinal biopsies seem to respond to major microbiota disruptions caused by acute GI GvHD or systemic antibiotic treatment. While observations in the upper GI tract seem to reflect Paneth cell damage, the relative increase in the lower GI tract may indicate inflammatory induction of AMPs in colonic epithelial cells in the course of GvHD.

[P718]



**Disclosure of conflict of interest:** None.

**P719**

**Results of stem cell transplantation in inherited bone marrow failure syndromes—single center experience**

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Inherited bone marrow failure (IBMF) syndromes are rare pediatric disorders that characteristically associate physical abnormalities, progressive bone marrow failure and predisposition to cancer. The most common of these disorders is Fanconi Anemia (FA). Stem cell transplantation (SCT) using related or unrelated donors are the only curative therapeutically approach when severe marrow failure is established. The aim of the study was to analyze the results of SCT for patients with IBMFS in a single center. We performed a retrospective study in pediatric patients with IBMF admitted in Pediatric Hematology and Bone Marrow Transplant Department, Fundeni Clinical Institute between January 2000 and September 2016. Diagnosis and severity of IBMFS were established based on hematological results, bone marrow biopsy and clinical findings. Genetic testing for IBMFS is not currently

available in our country. Indication for SCT was established when patients developed moderate/severe aplastic anemia and became transfusion dependent. In case of DBA, SCT indication was established for steroid resistant disease. The donors were selected from family members or unrelated donors, 10/10 matched. The conditioning regimens used were reduced intensity (Fludarabine 120–150 mg/m<sup>2</sup>, Cy 20 mg/kg, F-ATG 40 mg/kg) for AF, DC and myeloablative (Busulfan i.v., Fludarabine 150 mg/m<sup>2</sup>, Thiotepa 20 mg/kg, F-ATG 30 mg/kg) for DBA. GvHD prophylaxis consisted of standard Methotrexate and CSA/Tacrolimus. All parents signed informed consent forms. In our center, between 2000 and 2016, 20 patients with IBMF were diagnosed: 10 (50%) patients with FA, 6 (30%) patients with Diamond Blackfan Anemia (DBA), 2 (10%) patients with Diskeratosis congenita (DC), and 2 (10%) patients with not classifiable IBMFS. The patient data is available in Table 1. Seven out of 20 patients (35%) performed SCT procedures: sibling 3 patients (2 patients with AF, 1 patient with DC), MUD 4 patients (3 patients with AF, 1 patient with DBA). All patients (100%) engrafted for PMN (median = 17, range: 12–29 days) and platelet (median 21, range: 13–46 days). 2/7 (42%) presented reactivation of CMV and received Valganciclovir, 1/7 developed CMV disease (encephalitis and pneumonia), 2/7 (28%) developed BKV cystitis and required extensive hydration and Levofloxacin. 4/7 (57%) developed grade I–II skin acute GvHD day +100, which responded to topical treatment and low dose of corticosteroids. 1/7 (14%) developed grade III intestinal acute GvHD, which responded to high-dose corticosteroids. 1/7 (14%) developed grade IV intestinal chronic GvHD (day +160), without response to high-dose corticosteroids, MMF and later died on day +221, due to infectious complications (severe pulmonary and cerebral aspergillosis). 6/7 patients (85%) are alive, with 100% donor chimerism 4/6 (66%) or stable mixed chimerism 2/6 (33%). Median follow-up for SCT patients was 515 days (26 days–5y 6mo). Conclusions In our study we observed a low incidence of severe complications associated with low mortality rate (14%). SCT is a procedure that associates multiple risk situations, but it remains the only curative

[P719]

		IBMFS Patients	IBMFS patients with SCT
No.		20	8/20 (40%)
F:M		6:14	2:6
Age at diagnosis – Median (range)		5y 2mo (56 days - 14y)	5y 11mo (56 days – 12y 3mo)
Diagnosis	FA	10 (50%)	5/10 (50%)
	DBA	6 (30%)	1/6 (16%)
	DC	2 (10%)	1/2 (50%)
	not classifiable IBMFS	2 (10%)	1/2 (50%)
Time to transplant – Median (range)			646 days (235 days – 4y)
Follow-up – Median (range)			515 days (87 days – 5 y 6mo)

treatment for IBMFS syndrome, in context of 10/10 and RIC procedures.

**Disclosure of conflict of interest:** None.

## P720

### Safety and preliminary efficacy of "Ready to administer" cytomegalovirus (CMV)-specific T cells for the treatment of patients with refractory CMV infection

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Cytomegalovirus (CMV) infections remain a significant cause of morbidity and mortality in patients whose immune systems are compromised, including hematopoietic stem cell transplant (HSCT) recipients. Although the adoptive transfer of third party CMV-specific T cells has proven both safe and clinically beneficial in treating even drug-refractory infections/disease, broader implementation and commercialization of this strategy has been hampered by (i) the postulated need for extensive cell banks generated from donors representing diverse HLA profiles, and (ii) lack of large scale T cell manufacturing processes. To address these limitations we have developed a proprietary decision tool (Cytomatch™) to identify a small panel of healthy donors who should provide almost universal HLA coverage; and optimized a simple, scalable manufacturing process to generate large numbers of CMV-specific T cells. To assess the robustness of our strategy we generated a bank of CMV-specific T cells (Viralym-C™) from 8 carefully selected healthy donors. The lines were polyclonal, comprising both CD4<sup>+</sup> (21.3 ± 6.7%) and CD8<sup>+</sup> (74.8 ± 6.9%) T cells, expressed central CD45RO+/CD62L+ (58.5 ± 4.2%) and effector memory markers CD45RO+/CD62L- (35.3 ± 12.2%), and were specific for the immunodominant CMV antigens IE1 and pp65 (IE1: 419 ± 100; pp65 1070 ± 31 SFC/2 × 10<sup>5</sup>, n = 8). A fixed-dose (2 × 10<sup>7</sup> cells/m<sup>2</sup>) Phase I clinical trial was subsequently initiated to test the safety and efficacy of these "ready to administer" T cells in pediatric and adult HSCT recipients with drug-refractory CMV infections. Using our bank of just 8 lines, we have identified a suitable line for 21 of 22 patients screened. Of these, 7 patients have been treated with Viralym-C cells; 6 received a single infusion and 1 patient required 2 infusions for sustained benefit. There were no immediate infusion-related toxicities; and despite the HLA disparity between the Viralym-C™ lines and the patients infused, there were no cases of de novo or recurrent graft versus host disease (GvHD). Based on viral load (measured by quantitative PCR) and/or symptom resolution, Viralym-C cells controlled infections in all patients with 5 complete (CR) and 2 partial responses (PR) achieved within 4 weeks of infusion. One patient with CMV retinitis had complete resolution of symptoms following Viralym-C™ infusion. Our results demonstrate the feasibility, preliminary safety and efficacy of "ready to administer" Viralym-C™ cells that have been generated from a small panel of healthy, eligible CMV seropositive donors identified by our decision support tool. These data suggest that cost-effective, broadly applicable T cell anti-viral therapy may be feasible for patients following HSCT and potentially other conditions.

**Disclosure of conflict of interest:** Drs. Juan Vera, Ann Leen and Brett Giroir hold equity and Drs. Ifigeneia Tzannou, Sunitha Kakarla are employed by ViraCyte.

## P721

### Safety of dose escalating haploidentical memory T cell donor lymphocyte infusions after 45RA-depleted haplo-stem cell transplantation

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Haploidentical stem cell transplantation (HSCT) protocols utilizing *ex vivo* T-cell depleted grafts have been proven efficient in preventing graft versus host disease (GvHD), but cause a delay in early T-cell recovery that increases the risk of graft rejection, leukemia relapse and viral infections. Conventional donor lymphocyte infusion (DLI) after HSCT transplantation is conditioned because of the high prevalence of GvHD even with low dose of T cells. Here we present preliminary data of escalating CD45RO+ memory T cells as DLI in three patients that received a selective graft depleted of naïve (CD45RA+) T-cells. Three children that were transplanted following nonmyeloablative conditioning regimen with a graft consisting of CD34+ and CD45RA- cells, with mixed chimerism, lymphopenic and viral/opportunistic infections and minimal residual disease positive before HSCT received dose escalating cryopreserved haploidentical CD45RA- memory T cell starting with a initial dose of 1 × 10<sup>5</sup>/kg, until a maximal dose of 1 × 10<sup>8</sup>/kg with a 21 days interval. We infused 10 products with a naïve (45RA+) T-cell dose less than 1 × 10<sup>4</sup>/Kg with > 99.9% purity of CD3+ CD45RO+ memory T-cells in all cases. All infusions were well tolerated without any side effect during infusions neither GvHD. Following the DLI, a progressive increase in T cell counts was observed. Our preliminary data suggest that dose escalating of haploidentical memory T cells (45RO+) as DLI provides a safety platform, even with high dose of T cells (1 × 10<sup>8</sup>/kg), for adoptive immunotherapy in haploidentical 45RA+ depleted grafts with no GvHD complications, and allows an increase in T cell reconstitution. However, efficacy of this strategy requires longer studies.

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**Disclosure of conflict of interest:** None.

## P722

### Second haploidentical stem cell transplantation after relapse following first allogeneic transplantation

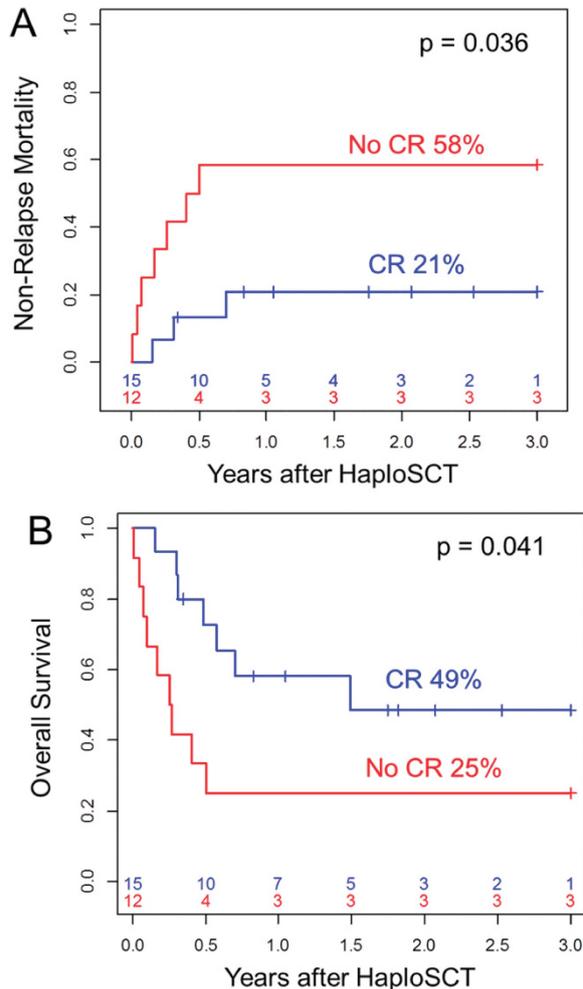
S Harbi, V Lavoipierre, L Castagna, A Granata, S Furst, C Faucher, F Legrand, S Bramanti, C Chabannon, N Vey, J Rey, R Bouabdallah, JM Schiano, D Mokart, PJ Weiller, D Blaise and R Devillier

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Relapse after allogeneic hematopoietic stem cell transplantation (alloHSCT) remains a major therapeutic challenge: outcome is very poor, without curative option in most cases. Second alloHSCT may be considered in few selected patients because of anticipated limitations: (1) donor availability; (2) high toxicity due to previous treatments; (3) low efficacy considering the very advanced disease situation. We hypothesized that the use of post transplantation Cyclophosphamide (pCY) haplo-SCT after relapse following alloHSCT may deal in part with these limitations. In particular, the presence of full haplotype HLA mismatch could provide a decisive antileukemic effect relative to alloreactivity. In absence of large series in this setting, we report here the outcome after HaploSCT for patients who relapse after a first alloHSCT. We retrospectively studied adult patients, who received a second pCY Haplo-SCT for hematological malignancies. Patients were treated between 2009 and 2016. The objective was to assess both the feasibility and the efficacy of HaploSCT in this setting. Twenty seven patients were included: median time between first alloHSCT and relapse was 11 months (range: 1–82). Median age at second transplantation was 49 years old (range: 21–61). Most of patients had acute myeloid leukemia (n = 12, 44%) or Hodgkin lymphoma (n = 6 patients, 22%). Fifteen

patients (55%) were in complete remission at the time of pCY Haplo-SCT. Hematopoietic cell transplantation-comorbidity index was  $\geq 3$  in 20 patients (74%). Thirteen patients (48%) received non-myeloablative conditioning regimen (as Baltimore schema, Luznik et al. BBMT 2008) prior to HaploSCT while remaining patients received busulfan-based regimen. All patients were given pCy and both CSA and MMF as GVHD prophylaxis. Day+100 cumulative incidence of grade 2 to 4 and 3 to 4 acute GVHD was 15% and 7%. 2-year cumulative incidence of chronic GVHD was 12%. The cumulative incidence of non-relapse mortality and relapse at 2 years were 38% and 27%, respectively. With a median follow up of 25 months (range: 4–63), 2-year progression-free and overall survivals were 36% and 39%, respectively. Disease status at the time of HaploSCT was a major determinant for outcome. Indeed, 2-year NRM and OS were 58% and 25% in patients transplanted with active disease, respectively, while corresponding values in patients transplanted in CR were 21% ( $P=0.036$ ) and 49% ( $P=0.041$ ), respectively (Figure 1A and 1B). We can conclude that in selected patients who could be candidate for second transplantation, HaploSCT is feasible and may represent a curative option. The overall incidence of relapse of 27% is promising in this situation for which no alternative for cure is available, with relatively good survival in patients transplanted in CR. However, the very high NRM (58%) in refractory patients should make us consider second transplant with caution in this setting. For these patients, specific developments are needed to avoid procedure-related toxicity.

[P722]



Disclosure of conflict of interest: None.

### P723 Secondary solid tumors following hematopoietic cell transplantation for thalassemia major

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Secondary solid tumors (SST) have been described after HCT, in particular for patients affected by hematologic malignancies. There is limited information about the incidence of SST following HCT for thalassemia major (TM). The aim of this study was to determine the incidence of SST in 134 patients with TM who received HCT in our Center between 1983 and 2013. 117 patients survived more than 3 years after HCT and were enrolled in the study. Of them, 57 were males and 60 females. Their median age at time of HCT was 10 years (1–29). As conditioning regimen, they received Busulfan (14 mg/Kg) and Cyclophosphamide (200 mg/Kg). The GvHD prophylaxis included Cyclosporine and Methotrexate. All patients received bone marrow cells from an HLA identical donor. At time of this report, 112 patients were cured, whereas 5 patients rejected their graft and are now under regular transfusion treatment. Overall, the median follow-up after HCT was 24 years (3–34). Seven patients developed a malignancy 3.2 to 28 years (median 16.4 years) after HCT including 2 carcinomas of the tongue, 1 oral squamous cell carcinoma, 1 colorectal cancer, 1 thyroid carcinoma, 1 carcinoma of the uterine cervix, and 1 parotid carcinoma. The 30-yr cumulative incidence (CI) of developing SST was  $10 \pm 0.17\%$ . All patients underwent surgical resection of the tumor and in addition 4 of them received chemotherapy and/or radiotherapy. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GvHD with buccal cavity involvement. 2 patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We compared these results with 2 case control populations. First of all, we investigated the occurrence of solid tumors in the 117 individuals (64 males, median age 10 years at time of marrow donation), who served as stem cell donors for HCT. One donor developed breast cancer 29 years after marrow donation at age of 38. The 30-yr CI of developing solid tumor for donors was  $4.5 \pm 0.21\%$  with a statistically significant difference ( $P=0.03$ ) as compared to that of transplanted patients. The second case control population consisted of 117 patients affected by TM treated with transfusions and iron chelation. The matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. 2 patients developed an hepatocellular carcinoma (HCC) at age of 39 and 44 years, respectively. One patient died and one is living. Using the event rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group ( $P=0.106$ ). This study shows that the magnitude of increased risk of SST is twofold to threefold for patients treated with HCT as compared with an age- and sex matched nontransplant TM patients or with stem cell donors. Notably, among the transplanted patients we didn't observe any case of HCC, which is one of the most frequent solid tumor in nontransplant TM patients, whereas we observed 4 cases of head/neck cancers. In our series, cGvHD seems to be a strong risk factor in the development of new solid tumors. Patients with cGvHD, especially those with involvement of the oral cavity, must receive a very long careful monitoring and surveillance in order to prevent the development of secondary cancers.

Disclosure of conflict of interest: None.

P724

**Sequential treatment with bortezomib plus thalidomide plus dexamethasone followed by autologous hematopoietic stem cell transplantation (HSCT); consolidation and maintenance therapy in patients with multiple myeloma**

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The management of Multiple myeloma (MM) has been significantly improved in recent years in young patients, where AHSCT and advent of new molecules was introduced as first line treatment. The sequential treatment (induction followed by autologous hematopoietic stem cell transplantation; consolidation and maintenance therapy) has increased rates response (CR and VGPR) as well as the overall survival. Our purpose was to assess the efficacy and adverse effects of sequential treatment with VTD chemotherapy and autologous HSCT followed by consolidation and maintenance therapy. In a prospective multicenter study, we evaluated this MM management strategy at Oran, in two hematology centers. Patients aged under 65 years with de novo MM, were treated with induction included: bortezomib (1.3 mg/m<sup>2</sup>, D1-D4-D8-D11), thalidomide (100 mg/m<sup>2</sup> D1-D21) and dexamethasone (40 mg, D1-D4; D8-D11). A total of 3 to 4 cycles were delivered every 21 days. Autologous stem cell was mobilized using G-CSF alone (15 µg/kg/day for 5 days). Leukapheresis to harvest stem cells were performed on day -2 and -1. The conditioning regimen consisted of melphalan 200 mg/m<sup>2</sup>. A consolidation phase was initiated two months later with the same protocol (VTD), followed by a maintenance treatment with thalidomide 50 mg/day given orally for 12 months. This study was done over a 6-years period (January 2010–December 2015). Fifty patients were included. They include 19 women and 31 men (sex ratio = 1.63). The median age at diagnosis was 53 years (32–64). According to Durie Salmon staging, 80% of patients were in stage III, while 38% were in stages III according to ISS staging. The monoclonal component was IgG in 56% of patients. Postinduction overall response rate in the 50 eligible patients was 100%, including 52% VGPR and 38% CR/ and 10% PR rates. The median of CD34 + rate was 3.88x10<sup>5</sup>/kg (1.41 to 11). All patients had engraftment on the median of day 10 (range; 7 to 14) and platelet transfusion independence on the median of day 13 (range; 9 to 19). There was no graft failure. One patient died following the procedure (TRM). Posttransplantation on day 100, CR and at least VGPR remained significantly higher (98%). In the 49 evaluable patients, the estimated OS at 79 months was 82%, the estimated DFS at 43 months was 66% and the PFS at 78 months was 66.5%. At the 30/09/2016, 43 (86%) patients are alive and 39 (80%) without disease activity after a median follow-up of 33 months (range; 3–79). The main hematological toxicities post transplant (Grade 3/4) were thrombocytopenia (49%), neutropenia (50%), and anemia (8%). The most frequently observed nonhematological toxicities (all grades) included peripheral neuropathy (66%). Our experience suggests that the sequential protocol used in first line produce a better outcome with fewer adverse events and is an interesting therapeutic option in term of efficacy and tolerance.

**Disclosure of conflict of interest:** None.

P725

**Serum and extracellular vesicle MicroRNAs miR-423, miR-199 and miR-93\* as biomarkers for acute graft-versus-host disease**

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MicroRNAs are small, non-coding single-stranded RNAs and regulate approximately 50% of all genes by repressing translation. They are present in bodily fluids, where they are protected from RNase-mediated degradation by encapsulation into extracellular vesicles (EVs) and demonstrate a novel capacity to regulate the cellular differentiation of blood cells and immune function. Candidate microRNAs miR-377, miR-199, miR-93\* and miR-423 have previously been associated with acute graft versus host disease (aGvHD) in post-hematopoietic stem cell transplant (HSCT) patient plasma. However, validation in independent cohorts is necessary, and their presence within extracellular vesicles (EVs) has not been explored. MicroRNA expression was evaluated in a prognostic cohort ( $n=81$ ) of day 14 (D14) post-HSCT patient serum samples by TaqMan qRT-PCR. Further assessment in an independent cohort of serum samples taken at the time of aGvHD diagnosis was also performed. Expression was also assessed in serum EVs at sequential time points (pre-HSCT, D0, D7 and D14) and an independent verification cohort of D14 serum samples by EV isolation, RNA extraction and TaqMan qRT-PCR analysis. This study replicated elevated serum expression of miR-423 ( $P < 0.001$ ), miR-199 ( $P=0.04$ ), miR-93\* ( $P < 0.001$ ) and miR-377 ( $P=0.03$ ) in aGvHD, in a prognostic cohort of D14 post-HSCT patient samples ( $n=81$ ). Expression was also associated with disease severity. Further analysis at aGvHD diagnosis in an independent cohort ( $n=65$ ) confirmed high expression of miR-423 ( $P=0.02$ ), miR-199 ( $P=0.007$ ) and miR-93\* ( $P=0.004$ ) at disease onset. Investigation of microRNA expression patterns during early HSCT at sequential time points (pre-HSCT to D28) identified elevated microRNAs at D7 post-HSCT in all transplant patients. In a novel investigation of microRNA expression in serum EVs ( $n=15$ ), miR-423 ( $P=0.09$ ), miR-199 ( $P=0.008$ ) and miR-93\* ( $P=0.001$ ) levels were lower at D14 in patients who later developed aGvHD, and this was replicated for miR-423 ( $P=0.02$ ) and miR-199 ( $P=0.04$ ) ( $n=47$ ). Comparing serum to circulating EVs, at D14 patients remaining aGvHD-free had significantly higher expression of miR-423 ( $P=0.03$ ), miR-199 ( $P < 0.001$ ) and miR-93\* ( $P=0.001$ ) in the EV fraction. Results validate the capacity for circulating serum miR-423, miR-199 and miR-93\* to act as diagnostic and prognostic biomarkers for aGvHD. Novel findings of differential expression between whole serum and the EV compartment prior to disease onset suggest a role for EV microRNAs in the biology of aGvHD, which warrants further investigation.

**Disclosure of conflict of interest:** None.

P726

**Simplified validated criteria for donor selection in acute leukaemia**

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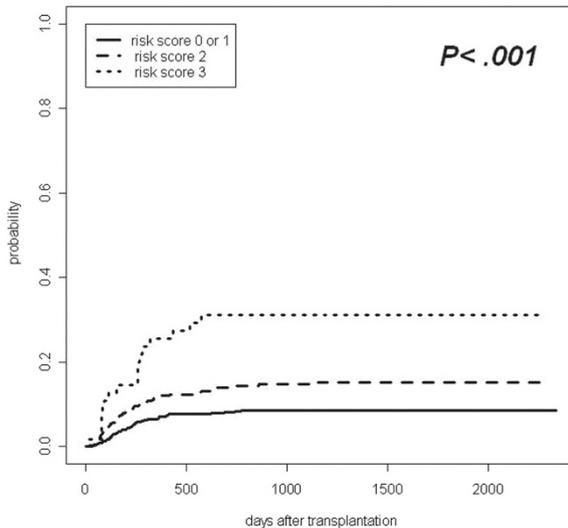
Prior data indicate similar outcomes after transplants from HLA-haplotype-matched relatives, HLA-identical siblings and HLA-matched unrelated donors. We used our dataset to answer a clinically important question: who is the best donor for a person with acute leukemia. We analyzed data from persons with acute leukaemia in 1<sup>st</sup> complete remission treated in a prospective, multi-centre study. Patients were randomly divided into training ( $n=611$ ) and validation ( $n=588$ ) sets. 1199 consecutive subjects received a transplant

from an HLA-haplotype-matched relative ( $N=685$ ) or an HLA-identical sibling ( $N=514$ ). 3-year leukaemia-free survivals (LFSs) were 75% (95% confidence interval [CI], 72, 78%) and 74% (70, 78%;  $P=0.95$ ). The multivariate model identified 3 major risk factors for transplant-related-mortality (TRM): older donor/recipient age (donor > 40 years/recipient > 30 years; Hazard Ratio [HR] = 1.88; [1.05, 3.35];  $P=0.03$ ), female-to-male transplants (HR = 2.11; [1.50, 2.98];  $P=0.01$ ) and donor-recipient ABO major-mismatch transplants (HR = 1.55 [1.08, 2.23];  $P=0.02$ ). A risk score was developed based on these three features. TRMs were 8% (5, 10%), 15% (12, 18%) and 31% (19, 43%) for subjects with scores of 0–1, 2 and 3 ( $P < 0.001$ ). 3 year LFS were 78% (75, 81%), 74% (70, 78%), and 58% (45, 71%;  $P=0.003$ ). The risk score was validated in an independent cohort. In recipients > 50 years, LFSs were 69% and 86% ( $P=0.08$ ) after transplants from identical-sibling or children. Our data confirm donor source or degree of HLA-disparity is not significantly correlated with transplant outcomes. Selection of the best donor needs to consider donor-recipient age, sex-matching and ABO-incompatibility amongst persons with acute leukemia receiving transplants from family members. [P726]

Table 1: Outcomes according to risk score.

Score	N	Transplant related mortality (TRM)			Overall survival	
		RR (CI)	P	3y% (CI)	3y% (CI)	Overall P
<b>Training set (n=611)</b>						
0-1	382	1.00	-	7 (4-10)	81 (77-85)	
2	201	2.10(1.22-3.62)	0.007	14 (9-19)	78(72-84)	
3	28	7.13(3.50-14.50)	<0.001	40 (22-58)	53 (35-71)	
<b>Validation set (n=588)</b>						
0-1	352	1.00	-	10 (7-13)	80(76-84)	0.04
2-3	236	1.64(1.04-2.59)	0.03	16 (11-21)	72(66-78)	

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Disclosure of conflict of interest: None.

P727

**Synergistic effect of KIR ligands missing and cytomegalovirus reactivation in improving outcomes of haematopoietic stem cell transplantation for treatment of myeloid malignancies**

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The lack of one or more HLA class I alleles, whose protein products are the ligands for KIR receptors, has been exploited as a prognostic factor for the outcome of patients with

haematological malignancies treated by haematopoietic stem cell transplantation (HSCT). Although it has been accepted that KIR-HLA interactions may influence the outcome of the HLA-mismatched HSCT, there is no consensus regarding the settings of HLA-matched transplantation. There are studies that have reported either benefits, or no effects, under the influence of inhibitory KIR-HLA interactions. Additionally, certain activating KIRs and/or reactivation of cytomegalovirus (CMV) infection have been reported to affect the outcome of HLA-matched transplantation. The goal of this study was to evaluate the influence of KIR-HLA genotypes on the outcome of patients undergoing treatment for haematological malignancies by non-T-depleted lymphocyte haematopoietic stem cell transplantation (HSCT) from HLA-matched sibling donors. The prospective study was conducted at the Center of Hematology, University of Campinas, and 50 patients and their donors were followed up from 2008 to 2014. KIR and HLA class I genes were genotyped and patients grouped based on the presence of KIR ligands combined with KIR genotype of their respective donors. Patients with all KIR ligands present ( $n=13$ ) had a significantly higher ( $P=0.04$ ) incidence of acute graft-versus-host-disease (GVHD) than patients with one or more KIR ligands missing ( $n=37$ ). The overall survival following transplantation of patients with myeloid malignancies ( $n=27$ ) was significantly higher ( $P=0.035$ ) in the group with one or more KIR ligands missing ( $n=18$ ) than in the group with all ligands present ( $n=9$ ). Presence of KIR2DS2 was associated with a worsening of HSCT outcome while reactivation of cytomegalovirus (CMV) infection improved the outcome of patients with one or more KIR ligands missing. Our results indicate that KIR-HLA interactions affect the outcome of the HLA-matched transplantation, particularly in patients with myeloid malignancies.

Disclosure of conflict of interest: None.

P728

**The impact of minimal residual disease and its kinetics prior to different types of allogeneic hematopoietic stem cell transplantation on clinical outcomes in patients with acute myeloid leukemia**

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This study investigated the impact of minimal residual disease (MRD) and its kinetics prior to different types of allogeneic hematopoietic stem cell transplantation (HSCT) on clinical outcomes in patients with acute myeloid leukemia (AML) in complete remission ( $n=132$ ). 107 patients who received unmanipulated haploidentical HSCT and 25 patients who received HLA-matched sibling HSCT were enrolled. MRD measured using 8-color flow cytometry (FCM) at fixed time points before transplantation was retrospectively analyzed. The patients were divided into four groups based on MRD kinetics before transplantation: consistent negative, positive to negative, negative to positive and consistent positive. During the follow-up, total twenty (15.2%) patients underwent relapse. Through unique variate analysis, none of MRD status at various time points before unmanipulated haploidentical transplantation was associated with clinical outcomes, as well as the dynamic change of MRD before HSCT ( $P > 0.05$ ), although the patients with consistent positive MRD before HSCT seemed to have a relatively higher incidence of relapse ( $P=0.151$ ). One-year cumulative incidence of relapse (CIR) were  $11.2 \pm 4.7\%$  vs.  $31.1 \pm 13.8\%$  in MRD consistent negative and consistent positive groups, respectively ( $P=0.202$ ). However, patients with positive MRD after the second chemotherapy or pre-MRD before HLA-matched sibling HSCT showed a significant poor outcomes including higher CIR ( $P=0.015$  and

$P=0.015$ ), lower disease-free survival ( $P=0.015$  and  $P=0.015$ ) and lower overall survival ( $P=0.01$  and  $P=0.014$ ). One-year CIR of the above two groups were  $5.6 \pm 5.4\%$  vs.  $57.1 \pm 18.7\%$  in MRD negative and positive patients, respectively ( $P=0.001$ ). In addition, those who had consistent positive MRD prior to HLA-matched sibling HSCT showed even worse outcomes compared to patients without pre-MRD. Unmanipulated haplo-identical HSCT might have the stronger graft-versus-leukemia effect compared to HLA-matched sibling HSCT. It suggested that those who received unmanipulated haploidentical HSCT with pre-MRD might not need more intensive relapse intervention after transplantation.

**Disclosure of conflict of interest:** None.

#### P729

##### **The retrospective study of allogeneic hematopoietic cell transplantation for 36 patients with mixed-phenotype acute leukemia in Toranomon Hospital, Japan**

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Mixed phenotype acute leukemia (MPAL) is a distinct clinical entity which was defined by World Health Organization (WHO) in 2008. Several groups have demonstrated the outcomes of allogeneic hematopoietic cell transplantation (HCT), and almost patients were in complete remission (CR) at the time of transplantation. In the real clinical setting, however, there are substantial number of patients who can not achieve CR after chemotherapy. We conducted a retrospective study including such patients to elucidate the outcome of allogeneic HCT in Toranomon Hospital, Japan. We studied the patients with MPAL diagnosed from July 2008 to September 2015. MPAL was diagnosed according to WHO classification in 2008. From June 2013, we examined cytoplasmic myelo-peroxydase (cMPO) routinely for flowcytometric analysis in all the patients, to distinguish MPAL from acute lymphoblastic leukemia (ALL). We included the patients who were diagnosed as MPAL in Toranomon Hospital, regardless of their diagnosis or clinical course in the previous hospitals. A total of 36 MPAL patients underwent their first allogeneic HCT with related bone marrow or peripheral blood stem cells (R-BM/PB) ( $n=9$ ), unrelated bone marrow (U-BM) ( $n=6$ ), and unrelated umbilical cord blood (U-CB) ( $n=21$ ). The median patient age was 41 years (range:17–69). The immunophenotype of leukemia cells included 23 cases of B and myeloid (B/MY) (64%) and 13 cases of T and myeloid (T/MY) cell lineage(36%).Eleven patients(31%) harbored Philadelphia chromosome. The remission induction chemotherapy was performed with ALL-type regimens in 31 patients, and acute myeloid leukemia (AML)-type regimens in 5 of 36 patients, 18 patients(50%) were not in CR at the time of transplantation. Myeloablative conditioning (MAC) regimens were used in 30 patients(83%). The 2-year overall survival (OS) rate was 43.1% (95% confidence interval (CI), 25.7–59.4%). To identify the factors that influenced OS, we performed univariate analysis and compared the following pre-transplantation factors: age at the time of transplantation ( $< 41$  vs.  $\geq 41$  years), committed immunophenotype (B/MY vs.T/MY), karyotype (Philadelphia chromosome (Ph vs.non-Ph), disease status at the time of transplantation (CR vs.non-CR), donor cell source (R-BM/PB vs.U-BM vs.U-CB, CB vs.non-CB), and conditioning regimen (MAC vs.reduced intensity conditioning). CR at the time of transplantation was extracted as a significant predictive factor for the better OS(2-year OS; CR vs. non-CR, 63.0% (95% CI, 32.1-82.8%) vs.22.2% (95% CI, 6.9–42.9%),  $P=0.009$ ). The cumulative incidence of relapse rate (RR) at 2 years after transplantation was 53.3% (95% CI, 31.8–70.8%). To identify the factors that influenced relapse rate, we

performed univariate analysis and compared pre-transplantation factors same as above. Harboring Philadelphia chromosome was extracted as a significant predictive factor for lower relapse rate (2-year RR; Ph vs.non-Ph, 27.3%(95% CI, 0.1–77.3%) vs. 66.5%(95% CI, 41.4–82.8%),  $P=0.003$ ). The older patients( $P=0.07$ ) and the patients in CR ( $P=0.05$ ) also showed a trend towards lower relapse rate. Allogeneic HCT provided 63.0% of 2-year OS for MPAL patients in CR at the time of transplantation. On the other hand, for patients not in CR, 2-year OS was approximately 20%. The use of tyrosine kinase inhibitors along with chemotherapy before transplantation might prevent relapse after transplantation in MPAL patients with Ph chromosome.

**Disclosure of conflict of interest:** None.

#### P730

##### **The role of time gap between first and second allogeneic hematopoietic stem cells transplantations and the effect of donor change in second transplant outcomes in patients with hematological malignancies**

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a standard of treatment for many patients with hematological malignancies. However, the disease relapse and graft failure after first allo-HSCT (1<sup>st</sup> allo-HSCT) lead to poor outcomes almost in all cases. Second allo-HSCT (2<sup>nd</sup> allo-HSCT) is one of primary options that can decrease the mortality in this group of patients. Here we report our experience of 15 patients who underwent 2<sup>nd</sup> allo-HSCT. The aim of the study was to estimate a clinical efficiency and practicability of 2<sup>nd</sup> allo-HSCT. We included 15 patients (9 males/6 females) with acute myeloid leukemia (AML,  $n=10$ ), acute lymphoblastic leukemia (ALL,  $n=3$ ) and myeloproliferative disease (MPD,  $n=2$ ) who underwent 2<sup>nd</sup> allo-HSCT for relapse (66,7%) or graft failure (33,3%) from the same ( $n=8$ ) or another donor ( $n=7$ ) between November 2012 and October 2016. Median age was 31 years (range: 18–42 years). Three (20%) patients had a matched related donor (MRD), nine (60%) patients had a matched unrelated donor (MUD) and three (20%) patients had a mismatched unrelated (MMUD) at the second transplant. To evaluate time gap affecting outcomes all patients were divided into two groups: who underwent 2<sup>nd</sup> allo-HSCT in more/less than 6 months after 1<sup>st</sup> allo-HSCT. In “less than 6 months” group three patients were re-transplanted for relapse and one-for graft failure, in other group there were seven patients who received 2<sup>nd</sup> allo-HSCT for disease relapse and four-for graft failure. Fisher’s exact test were performed to exclude probability of imbalance between groups ( $P>0.05$ ). Median of overall survival (OS) and disease-free survival (DFS) after 2<sup>nd</sup> allo-HSCT was 13.5 months and 10.59 months respectively. (See Figure 1A,1C) Two patients (13.3%) developed graft failure and three relapsed (20%). Acute graft-versus host disease (aGVHD) incidence was extremely low as 13.3% ( $n=2$ ) even despite use of MUD/MMUD in 80% of cases. Mortality rate were 53.3% in a group of 2<sup>nd</sup> allo-HSCT. It should be noted that only 3 (20%) patients died because of disease progression. Five patients (33.3%) died in complete remission due to severe infections or previous toxicity (e.g. heart failure). The effect of donor change on DFS was not significant ( $P=0,88$ ). Our statistical analysis reveal significantly differences in OS in patient with long-term interval ( $> 6$  months) between 1<sup>st</sup> and 2<sup>nd</sup> allo-HSCT. Median of OS in patients who underwent 2<sup>nd</sup> allo-HSCT in more/less than 6 months after 1<sup>st</sup> allo-HSCT was 20,59 vs 7,02 months respectively. (See Figure 1B,1D) For hazard ratio (HR) estimation Mantel-Haneszel approach were used HR for group who were transplanted in less than 6 months from 1<sup>st</sup> allo-HSCT was 8.36, (95% CI, 1.054

to 66.38,  $P=0.04$ ). As for DFS difference was not significant ( $P=0.07$ ).

According to our analysis, performing 2<sup>nd</sup> allo-HSCT in a period less than 6 months after 1<sup>st</sup> allo-HSCT seemed not very reasonable due to extremely high mortality even in young patients (HR=8.36,  $P=0.04$ ). As for “more than 6 months” group it can be considered even despite HLA-disparity between donor-recipient pair due to extremely low aGVHD rate (13.3%). Donor change was not associated with better outcome ( $P=0.88$ )

**Disclosure of conflict of interest:** None.

**P731**

**The start-up of the first HSCT center in the Iraqi Kurdistan: A cooperative project by the Hiwa cancer hospital, Sulaymaniyah, and the Italian agency for development cooperation**

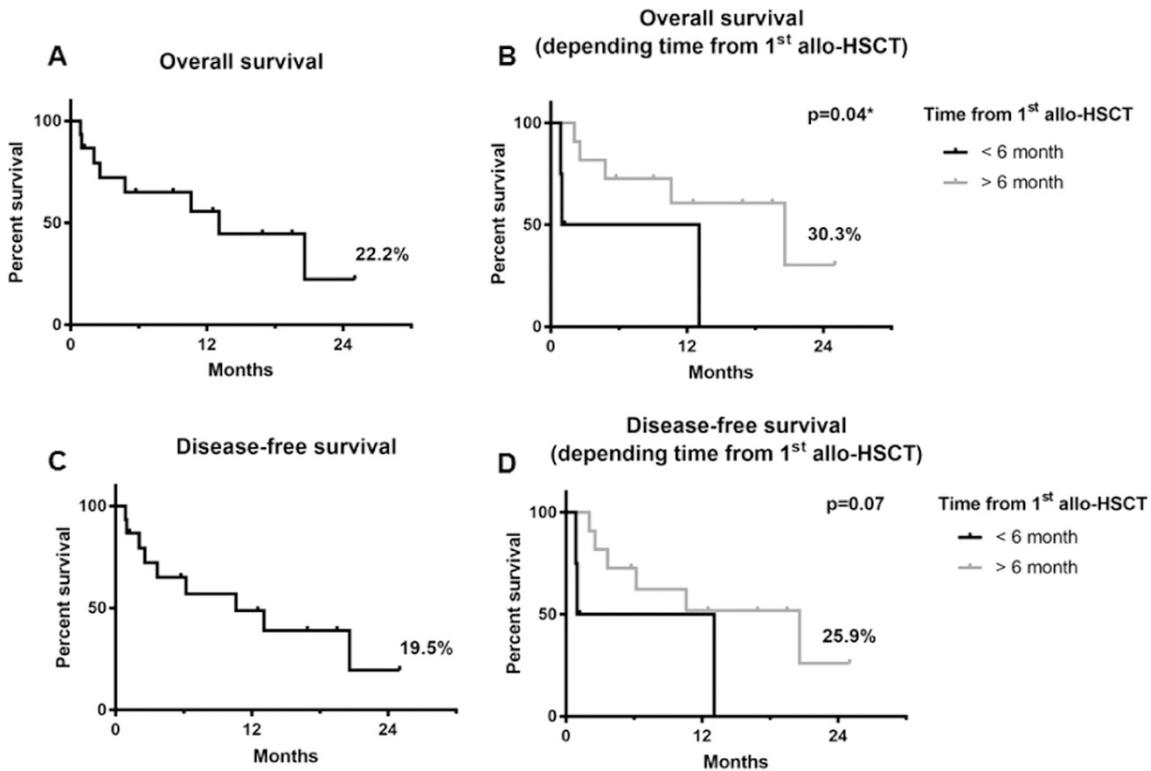
I Majolino<sup>1</sup>, D Othman<sup>2</sup>, A Rovelli<sup>3</sup>, D Hassan<sup>2</sup>, L Rasool<sup>2</sup>, M Vacca<sup>1</sup>, N Abdalrahman<sup>2</sup>, C Abdullah, Z Ahmed<sup>2</sup>, D Ali<sup>2</sup>, K Ali<sup>2</sup>, C Broggi<sup>1</sup>, C Calabretta<sup>1</sup>, M Canesi<sup>1</sup>, G Ciabatti<sup>1</sup>, C Del Fante<sup>1</sup>, E De Sapia<sup>1</sup>, G Dore<sup>1</sup>, A Frigato<sup>1</sup>, M Gabriel<sup>1</sup>, C Girmenia<sup>1</sup>, F Ipsevich<sup>1</sup>, H Kareem, D Karim, N Khoshnaw<sup>2</sup>, R Leone<sup>1</sup>, T Mahmood, Manna<sup>1</sup>, MS Massei<sup>1</sup>, A Mastroia<sup>1</sup>, D Mohammed, D Noori, A Ostuni<sup>1</sup>, A Palmas<sup>1</sup>, M Possenti<sup>1</sup>, A Qadir, G Real<sup>1</sup>, H Sadiq, R Shrif, C Valdatta<sup>1</sup>, S Vasta<sup>1</sup>, M Verna<sup>1</sup>, M Vittori<sup>1</sup>, A Yousif<sup>2</sup>, F Zallio<sup>1</sup> and S Quattrocchi<sup>4</sup>

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Hemopoietic stem cell transplantation (HSCT) is an effective treatment for many hematologic disorders, and globally over 70 000 procedures/year are performed in more than 70 countries. However, not all the countries have enough

resources and expertise to establish an HSCT program, and patients are often forced to emigrate for transplantation, with heavy social and economic consequences. In the year 2015 the IUC (an Italian NGO) identified the Hiwa Cancer Hospital (HCH) in Sulaymaniya (Iraqi Kurdistan) as a possible site for the establishment of a new HSCT transplant center. A HSCT expert from Italy (MI) following a visit to the HCH, reported a positive conclusion on the feasibility of an HSCT project. This was mainly due to the fact that many of the required technologies were already available at HCH, including a 6-bed positive-pressure, HEPA-filtered-air clinical unit, 2 last-generation cell separators and a well equipped HLA laboratory. Following this preliminary survey, a capacity building project was rapidly made and submitted to the Italian Agency for Development Cooperation, that approved and funded it in March 2016 with the specific aim to cure thalassemia patients either of Kurdistan and of the refugees population from Syria and other parts of Iraq. In April 2016, the joint Italian and kurdish team started the project. A first autologous transplant was done in June 2016 followed by 8 more autografts (overall, 5 myeloma and 4 lymphoma patients). In October, following appropriate downstaging, a first low-risk thalassemia patient was allografted from her HLA-id sibling, followed by 2 more patients. All the patients engrafted promptly, with one death occurring on day +23 with acute cardiac failure and a major toxicity recorded in a single patient (NHL, severe enterocolitis with perforation) that was successfully treated. The full process for the start-up included the following activities developed during 8-month time: (1) intensive course of lectures on site; (2) selection and training of dedicated nurses; (3) development of stem cell mobilization, collection and cryopreservation; (4) surgical CVC insertion training; (5) development of proper immune-hematology laboratory techniques; (6) implementation of blood irradiation and TBI; (7) building of an infection control program; (8) definition of a responsibility tree; (9) development of a clinical expertise, and (10) construction of a protocol book containing all the SOP. Following the first series

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of transplants, the HCH group also submitted to EBMT an application for full membership, that was promptly approved. In all this project, the Italian counterpart provided over 30 highly-experienced volunteer specialists (physicians, nurses, technicians and one physicist), each with a specific mission plan. Despite the many difficulties and obstacles encountered, the clinical results obtained so far appear encouraging, though there is still need to further support the HCH in order to make it totally independent. Following this intervention, the HCH is the only one center performing both auto and allo HSCT not only in the Iraqi Kurdistan Region, but also in all the Iraqi Nation. We conclude that international cooperation may be fruitful also in the field of high-technology medicine, and may contribute to improve the capabilities of centers even in critical geographic areas, representing a valuable instrument also to implement nation-to-nation scientific exchanges.

**Disclosure of conflict of interest:** None.

### P732

#### The use of plerixafor with G-CSF in conditioning regimen for hematopoietic stem cell transplantations with TCR alpha/beta and CD19 depletion of graft in Wiscott-Aldrich syndrome patients: A single-center experience

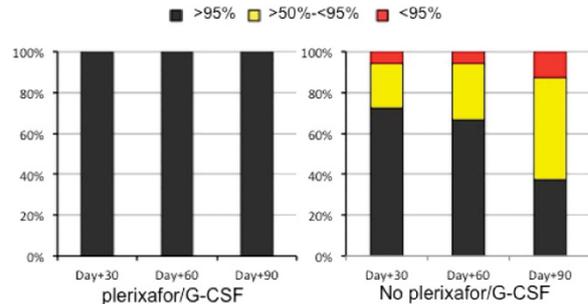
B Dmitry<sup>1</sup>, L Alexandra<sup>2</sup>, S Larisa<sup>1</sup>, G Elena<sup>1</sup>, S Irina<sup>1</sup>, T Pavel<sup>1</sup>, K Rimma<sup>1</sup>, N Galina<sup>3</sup>, M Michael<sup>1</sup> and M Alexei<sup>1</sup>

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High incidence of mixed chimerism with impaired graft function remains a significant issue in patients with Wiskott-Aldrich Syndrome (WAS) after HSCT. Simultaneous use of plerixafor with G-CSF is efficient in inducing stem cell release and opening of bone marrow niches. The use of plerixafor/G-CSF in conditioning demonstrates better levels of donor chimerism in patients with acute myeloid leukemia. We report our experience of plerixafor/G-CSF usage in patients with WAS as an addition to myeloablative conditioning to improve stem cell engraftment. From November 2012 to June 2016 18 WAS patients were transplanted in our center with TCRab/CD19 depleted grafts. For 11 patients Treosulfan 36–42g/m<sup>2</sup> + Fludarabine 150mg/m<sup>2</sup> regimen was used. Due to the high incidence of graft rejection 6 patients additionally received Melphalan 140 mg/m<sup>2</sup>. All but one patients received Thymoglobulin 5mg/kg and Rituximab 100mg/m<sup>2</sup>. One patient had Treosulfan + Fludarabine based conditioning with switching from Thymoglobuline to Cyclophosphamide 120mg/kg. 12 patients received grafts from unrelated (1–8/10; 3–9/10; 8–10/10 HLA matched) and 6 from haploidentical donors. Median age at HSCT was 2 years (0.9–12.6). OS was 0.89 (95% CI 0.74–1.0). However, EFS was 0.5 (95% CI 0.22–0.78) with no significant differences in patients with one and two alkylating agents in conditioning 0.5 (95% CI 0.29–0.86) vs 0.5 (95% CI 0.1–0.9), *P*=0.85. Events were considered: death in 2 patients, graft rejection in 5 patients, mixed myeloid chimerism (less than 20% donor) in 2 patients. Median time of event was 3,2 months after HSCT (1.23–8.6). From June 2016, a new approach for TCRab/CD19 depleted transplantation with additional Plerixafor and G-CSF in patient's conditioning regimen was performed for 9 WAS patients. Conditioning included Treosulfan 36–42g/m<sup>2</sup> + Fludarabine 150 mg/m<sup>2</sup> + Melphalan 140 mg/m<sup>2</sup> + Thymoglobulin 5 mg/kg + Rituximab 100 mg/m<sup>2</sup> + G-CSF 10 mg/kg days -8,-7,-6,-5,-4 + Plerixafor 240 mcg/kg days -6,-5,-4. 7 patients had first HSCT, 2 – second (due to previous graft rejection). For 3 patients MUD and for 6 - haploidentical donors were used. Median age at HSCT was 2,1 years (0,85–11,98). Median dose of infused CD34+ cells was 11.2x10<sup>6</sup>/kg (7.6–12.6), TCRab cells 21x10<sup>3</sup>/

kg (2–92). All patients after plerixafor/G-CSF-containing conditioning engrafted, median time of neutrophil engraftment was 12 days after HSCT (10–20), platelet 10 days after HSCT (9–16). All patients are alive, median FU is 3 months, range: 0.43–6.3. 2 patients had acute GVHD: 1–grade 2 (GUT), 1- grade 1 (skin), in both cases resolved after a short course of steroids. All patients had more than 95% donor chimerism monthly till the time of last FU. The comparison of peripheral blood chimerism (% of donor cells) in WAS patients transplanted with and without plerixafor/G-CSF in conditioning is shown (Figure 1). The additional use of plerixafor with G-CSF in conditioning looks like a promising solution to the prevention of graft failure in patients with WAS. Nevertheless, this approach needs further long-term investigation of safety and efficacy.

[P732]



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**Disclosure of conflict of interest:** None.

### P733

#### Therapeutic effects of autologous peripheral blood stem cell transplantation on advanced neuroblastoma and childhood rhabdomyosarcoma

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Both neuroblastoma (NRB) and rhabdomyosarcoma (RMS) in childhood are the aggressive malignant disease with higher mortality. This paper aims to study the efficacy of autologous peripheral blood stem cell transplantation (APBSCT) in the treatment of high risk advanced NRB and RMS. 34 patients with high-risk stage IV NRB and 9 patients with advanced childhood RMS were treated by APBSCT in our hospital from October of 2010 to May of 2016. In the subgroup of NRB patients, 16 patients got complete remission (CR) and 1 patient got CRu while 17 patients had tumor residual disease after intensive induction therapy before ASCT. The median age was 5.55 (1–9) years old. Primary sites of the tumors included submaxilla (*n*=2), cervical (*n*=5), adrenal gland (*n*=16) and retroperitoneal (*n*=11). The conditioning regimen consisted of busulfan and melphalan (busulfan 1 mg/kg × 4d, melphalan 140mg/m<sup>2</sup> × 1d) or CEM regimen (carboplatin 600 mg/m<sup>2</sup> × 3d, etoposide 500 mg/m<sup>2</sup> × 3d, cyclophosphamide 1800 mg/m<sup>2</sup> × 2d); The pathology of 9 stage III childhood RMS patients was embryonal rhabdomyosarcoma. There were 8 cases in CR and 1 case in partial remission (PR). The median age was 6.56 (3–13) years old. Primary sites of the tumors included bladder (*n*=1), left forearm (*n*=3), Retroperitoneal (*n*=1), pelvic (*n*=3)

and talus ( $n=1$ ). The conditioning regimen consisted of melphalan, cyclophosphamide and Dactinomycin (melphalan  $60 \text{ mg/m}^2 \times 3\text{d}$ , cyclophosphamide  $1800 \text{ mg/m}^2 \times 2\text{d}$ , dactinomycin  $0.013 \text{ mg/kg} \times 3\text{d}$ ). There were 13 double APBSCT cases (NRB  $n=12$ , RMS  $n=1$ ). All the relapse patients were treated with chemotherapy and radiation therapy. All the patients successfully underwent mobilization, collection and reinfusion. The time of hematopoietic reconstitution was  $(11.0 \pm 3)$  days, no severe toxicity was observed, no transplant-related death was found. With a median follow-up of 25.35(2–60) months, one of the patients was lost to follow-up. In the subgroup of NRB patients ( $n=33$ ): The 2-year event-free survival and total survival rate of all patients were 66.2% and 79.4%, respectively. The survival time of no recurrence was significantly different between the double transplantation group and single transplantation group ( $P < 0.05$ ). In the subgroup of RMS patients ( $n=9$ ), 1 patient died, 6 patients live without PD(1 patients had double APBSCT), 2 patients suffered recurrence but still alive. APBSCT achieved good outcome in patients with high risk advanced NRB and RMS. Transplantation-related toxicities were tolerable. Double APBSCT significantly improved the depth of remission.

**Disclosure of conflict of interest:** None.

#### P734

### Transplantation outcomes of a once-daily intravenous Busulfan and Fludarabine conditioning for Allogeneic hematopoietic stem cell transplantation in pediatric AML and high risk MDS: Single center experience in Korea

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There have recently been some reports suggesting that once-daily intravenous busulfan as a conditioning regimen for hematopoietic stem cell transplantation (HSCT) possibly reduces the toxicities without influencing the clinical outcome as compared with the traditional 4 times daily dosage schedule. But until recently there has been little research and limited data available on the safety and efficacy of once-daily intravenous busulfan and fludarabine in pediatric allogeneic HSCT. We report the outcomes for allogeneic HSC recipients, evaluating engraftment status, regimen related toxicities (RRT), and event free survivals (EFS) after use of once-daily intravenous busulfan and fludarabine conditioning for allogeneic HSCT in children with AML and high risk MDS in a single pediatric center of Korea. From January 2005 to December 2015, 22 AML and 2 high risk MDS children who received once daily iv busulfan/fludarabine based conditioning regimen for allogeneic HSCT were reviewed. Bu/Flu  $\pm$  ATG consist of intravenous fludarabine ( $40 \text{ mg/m}^2$ ) and busulfan ( $110 \sim 130 \text{ mg/m}^2$ , once daily iv) on days -6 to -3, and antithymocyte globulin (ATG) ( $3 \text{ mg/kg}$ ) on days -3 to -1. All patients received tacrolimus and mini-dose methotrexate ( $5 \text{ mg/m}^2$ ) for graft versus host disease (GVHD) prophylaxis. 17 boys and 9 girls were enrolled with median age of 10.1 years (range: 0.6–17.9 years). The median period from diagnosis to transplantation was 7 months (range: 5–49 months). More than half of the patients had a matched sibling donor ( $n=16$ , 62%), 27% patients ( $n=7$ ) had a matched unrelated donor, 8% patients ( $n=2$ ) had a mismatched unrelated donor, and the remaining 1 patient had a mismatched family donor. As a stem cell source, peripheral blood stem cells (PBSC) were 22 cases (85%), bone marrow and cord blood were 2 cases in each. The median follow-up for patients was 40 months. The median number of infused total nucleated cells and CD 34+ cells except cord blood transplantation were  $9.2 \times 10^8/\text{kg}$  and  $7.8 \times 10^6/\text{kg}$ . All patients including who received cord blood were successfully engrafted. The median time to absolute neutrophil count (ANC) recovery ( $\text{ANC} > 500 \times 10^6/\text{L}$ ) and platelet recovery (platelet  $> 20,000 \times 10^6/\text{L}$ ) were 13 days, 18 days in each. The incidence of acute GVHD was 19.2%, while severe grade III/IV GVHD was observed in only 2 patient

(7.7%). There were only two cases (8.7%) of extensive chronic GVHD in this study. Transplant-related toxicities were acceptable, there was no case with CNS toxicity, eleven patients (42.3%) developed grade II,III mucositis and grade I–III hepatic toxicity in twenty four (92.3%), but transient. There was 3 clinically diagnosed veno-occlusive disease (VOD), but most recovered by fluid restriction and diuretics. Nine patients (36%) showed positive cytomegalovirus (CMV) antigen/PCR but only one patient developed CMV colitis. Eight patients died: 7 due to relapse/disease progression, 1 due to extensive chronic GVHD. The 5-year EFS and overall survival were 62.2% and 66.1% respectively. At 3 year, the cumulative incidence of relapse was 19.2%. Overall, once-daily intravenous busulfan and fludarabine was less toxic and effective as conditioning regimen in AML and high risk MDS patients undergoing allogeneic transplantation in children.

**Disclosure of conflict of interest:** None.

#### P735

### Unmanipulated haploidentical hematopoietic stem cells transplantation (HaploSCT) with post-transplant cyclophosphamide: Experience of the hematology and stem cell transplant center of Pesaro, Italy

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Haploidentical Stem Cell Transplantation from unmanipulated graft has becoming a practiced option for high risk hematological malignancies who lack a matched related or unrelated donor. Lack of a matched sibling or unrelated donor (MUD) can be a significant barrier to allogeneic transplantation in patients who stand to benefit from this procedure. HLA-haploidentical donors are readily available for nearly all such patients. Haplo transplantation has inherent advantages over MUD transplantation including the lower cost of graft acquisition, greater availability of donors for ethnic minorities, and immediate access to the donor in patients in whom delay is undesirable. Fifteen pts (3 males, 12 females, median age 48, range: 17 – 65 years) with high risk hematologic malignancies ( Acute Myeloid Leukemia n.10, 66%; Acute Lymphoblastic Leukemia n 2, 13%; Chronic Lymphocytic Leukemia n.1, 6%; Multiple Myeloma n.2, 13%) received an unmanipulated bone marrow (BM) HaploSCT between November 2012 and November 2015. Donor-recipient pairs were typed at a molecular high resolution level at HLA-A, -B, -C and -DRB1 loci. HLA mm were defined at antigen level. Eight pts (53%) were in complete remission (26% CR1, 26% CR2), seven pts (47%) in advanced phase (CR3 and above, relapse or primary induction failure). Four patients (26%) had a previous allogeneic (2 pts, 13%) or autologous transplant (2 pts, 13%). Pretransplant conditioning regimen consisted of Thiotepa  $10 \text{ mg/kg}$  in two days, busulfan  $9.6 \text{ mg/kg}$  in three days, and Fludarabine. Source of stem cells was G-CSF stimulated bone marrow in all. Dose of marrow nucleated cells and CD34+ were  $5.4$  (range:  $3.4\text{--}6.7$ )  $\times 10^8/\text{kg}$  and  $3.5$  (range:  $2.1\text{--}5.8$ )  $\times 10^6/\text{kg}$  respectively. Post-transplant Cyclophosphamide at  $50 \text{ mg/kg/day}$  was given on days 3 and 5 after transplantation, together with Cyclosporine (starting at day -1 until day 180 post-transplant) and mycophenolate (from day +1 to day +20). CMV donor/host status was neg/neg for 13% of pts, pos/neg for 6%, neg/pos for 20%, pos/pos for 60%. Of the 15 donor-recipient pairs, 80% had 4 HLAmm on unshared haplotype (considering A,B,C, DRB1 loci), 20% had 3 HLAmm, 0% had 2 HLAmm. Overall, 14 pts (93%) achieved neutrophil engraftment at a median of 18 (range: 14–21) days. At day 100, incidence of

grade 2 acute GvHD (aGvHD) was 13% (2 pts). No pt experienced a grade >2 aGvHD. Three patients presented a limited form of chronic GvHD (21%). Incidence of oral mucositis and gastrointestinal/liver toxicity has been extremely low in this population of patients, even in those with active disease and heavily treated at the time of transplant. Eight out of fifteen pts (53%) are alive with a median follow-up of 31 months (range: 12 – 48 m). Seven (47%) are in cytogenetic/molecular remission. Six out the eight patients who were transplanted in CR1 or CR2 are alive (75%), while two out the seven patients who were transplanted in advanced phase are alive (29%). In this preliminary clinical experience, we find that unmanipulated haploidentical transplants with post-transplant Cyclophosphamide are a valid alternative and have outcome comparable to unrelated and match sibling transplants, in pts with hematologic malignancies. Advanced disease is the only adverse factor for disease-free survival. We therefore consider this therapeutic option when a match sibling or a 10/10 Ag MUD donor is not immediately available.

**Disclosure of conflict of interest:** None.

### P736

#### **Wharton's jelly-derived mesenchymal stem cells treatment in children with autism spectrum disorder: Our first preliminary results of the clinical application in Poland**

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impaired social communication and interactions with restricted and repetitive behaviors. Although ASD is suspected to have either heritable or sporadic genetic basis, its fundamental etiology and pathogenesis are poorly understood. Recently researchers have suggested that stem cells have therapeutic potential for ASD. Wharton's jelly-derived MSC (WJ-MS) from third-party donors (TPD) have high proliferation and differentiation potential. This cell population has also non-immunogenic and immunomodulatory properties, thus seem to be a promising treatment stem cell source. The Polish Stem Cell Bank (PBKM) has provided WJ-MS for clinical application in a medical therapeutic experiment for children with ASD. Twenty-three patients (pts) with ASD aged from 3 to 18. 10/12 (median age: 7 years and 7 months), after Bioethical Committee approval, received intravenous injections of WJ-MS, obtained from TPD. The cells were previously collected from healthy newborns, then processed, screened for bacterial contamination as well as endotoxin content, and frozen in liquid nitrogen vapour. WJ-MS immunophenotype was confirmed using flow cytometry assay. The pts received from 1 to 5 injections in intervals from 4 to 12 weeks. The average cell dose per infusion was  $1.01 \times 10^6$ /kg of body weight (bw). Each pt was examined by the same neurologist at the day of infusion. Comorbidities present in some patients: unspecified speech disturbances, flaccid paralysis, flaccid tetraplegia, unspecified encephalopathy, epilepsy, sensorineural hearing loss. One patient was diagnosed with 2 comorbidities: conductive hearing loss and intellectual disability. Almost 80% of pts, after their treatment with WJ-MS, revealed positive changes in neurological examination. An improvement in speech was observed in 10 pts and improvement of cognitive functions ensued in 7 pts. What is more, 26% of children showed progress in self-reliance, social interactions and improved their ability to concentrate. There was a reduction of aggressive behavior in 4 pts and 2 pts have

experienced better quality of sleep. There was only one adverse event after WJ-MS infusions - psychomotor agitation occurred in 24 hours after the administration. Five follow-ups have not yet been completed. The administration of third-party donor WJ-MS seems to be safe and efficient procedure with promising preliminary results in patients with ASD.

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**Disclosure of conflict of interest:** None.

### P737

#### **Wharton's jelly-derived mesenchymal stem cells treatment in children with cerebral palsy: Our second preliminary results of the clinical application in Poland**

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Cerebral Palsy (CP) is a heterogeneous group of conditions that result in permanent motor disability. It may occur due to perinatal hypoxic insults, developmental brain abnormalities, genetic diseases, traumatic or infectious causes. In general the condition is non-progressive, but improvement over time is rarely seen. Various treatment methods have been used for the management of this disorder. However, there has been no absolute cure for CP. The ultimate goal of stem cells therapy is to use the regenerative capacity of the stem cells causing a formation of new tissues to replace the damaged tissue. The Polish Stem Cell Bank (PBKM) has provided Wharton's jelly-derived MSC (WJ-MS) for medical therapeutic experiment application in children with CP. WJ-MS from third party donors were administered to 27 patients (pts) with CP aged from 1.6/12 to 16.9/12 (median age: 6 years and 1 month). Twenty two pts have received infusions intravenously (i.v.), 1 pt intrathecally (i.t.), and 4 pts via both routes (first i.v., next i.t.). The cells were previously collected from healthy newborns, processed, screened for bacterial contamination as well as endotoxin content, and frozen in liquid nitrogen vapour. MSC immunophenotype was confirmed using flow cytometry assay. The pts have received from 1 to 6 infusions in intervals from 4 weeks to 6 months. Median i.t. dose was  $15 \times 10^6$  cells per infusion, while median i.v. dose was  $1 \times 10^6$  cells/kg of body weight per infusion. Each patient has been examined by the same neurologist at the day of each infusion and the result of examination has been described in a follow-up. Twelve patients were diagnosed with epilepsy as comorbidity. Eighteen pts (67%) showed positive changes in neurological examination after their treatment with WJ-MS. Almost half of the children experienced improvement of cognitive functions (12 out of 27 pts). Muscle tension was reduced in 6 pts. Improvement in the ability to concentrate, better contact with others and improved social interactions were observed in 19% of pts. Correction of motility was noticed in 5 pts, 2 pts have experienced better quality of sleep. In 3 cases there has been a reduction in the number of epileptic seizures (1 pt even discontinued some of his medicines). There were no

noticeable changes in neurological examination of 2 patients. Seven follow-up forms have been not received yet. The experiment data provide evidence that third-party donor WJ-MSC are suitable and efficient stem cells for treatment in patients with CP. However further and more extensive examination, with a greater number of patients is needed, which will be beneficial for far-reaching results.

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**P738**

**Wharton's jelly-derived mesenchymal stem cells treatment in children with Spina bifida: Our first preliminary results of the clinical application in Poland**

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Spina bifida (SB) is a congenital malformation resulting from failure of fusion in the spinal neural tube during embryogenesis. Despite surgical repair of the defect, most patients who survive with spina bifida have multiple system damage due to neuron deficiency in the spinal cord. It has been confirmed that the mesenchymal stem cells (MSCs) have the ability to survive, migrate and differentiate into cells of a neural lineage. Wharton's jelly-derived MSCs (WJ-MSCs) from third-party donors have high proliferation and differentiation potential

along with non-immunogenic features, thus seem to be a promising stem cell source. The Polish Stem Cell Bank (PBKM) has provided WJ-MSC for clinical application in a medical therapeutic experiment for children with SB. Eleven patients (pts) were qualified for administration of WJ-MSCs. Three pts have been waiting so far for their therapy after Bioethical Committee approval. Seven pts were in the middle of stem cell therapy (after 1 or 2 injections), 1 pt had finished one cycle of stem cell therapy (5 injections - ijs) and resumed therapy by administering a first dose of WJ-MSCs. The cells were previously collected from healthy newborns, processed, screened for bacterial contamination as well as endotoxin content, and frozen in liquid nitrogen vapors. Six pts have received infusions intravenously (median dose:  $1.01 \times 10^6$ /kg body weight per infusion), and 1pt was given 1 injection of  $40 \times 10^6$  cells intrathecally. Each patient has been examined by the same neurologist at the day of each infusion and the result of examination has been written in a follow-up. There were 6 pts, who received at least 2 doses of WJ-MSC, and all of them showed positive changes in neurological examination. The important improvement, declared by pts, was in areas: pronunciation and/or self-reliance (3pts), movement of arms and/or legs (4pts), quality of life (3pts), core stabilization (1pt). Only one adverse event occurred after third injection of WJ-MSC: 1 pt had nausea and a fever. In case of other pts it was too early to provide reliable feedback. The transplantation of WJ-MSCs could stimulate the MSCs to differentiate towards sensory neurons. This could be one of the reasons of observed improvement of many vital functions in patients, after MSCs treatment. This approach might have value in the experimental treatment of sensory neuron deficiency in spina bifida.

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